ELECTROENCEPHALOGRAPHIC AND BIOCHEMICAL STUDIES OF TAURINE DURING INDUCED EPILEPTIFORM ACTIVITY IN RATS

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To Donna, Michael Jr. and Cathy

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By

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Chairman: Arnold H. Nevis
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The fundamental molecular basis of bioelectricity, and especially of the electroencephalogram (EEG), has been approached by a quantitative and correlative analysis of the role of taurine in suppressing electrical hyperactivity and associated epileptiform seizures in rats. Taurine was initially predicted to have such an anti-epileptic effect which was confirmed in the preliminary phases of this study, using quantitative electroencephalographic and behavioral indices of seizure activity. The seizure activity was controllably produced by osmotic agents. This dissertation describes the experimental relationship between the endogenous level of taurine in the rat brain and a quantitative measure of the abnormalities of the brain electrical activity during epileptic activity. The brain taurine concentration is measured at different times during the progress of epileptiform activity and these data are related to the excitability rating of the brain at the time of sacrifice.

It is shown that the brain taurine level does not vary significantly during the progress of the seizure. However there is shown to be a correlation between the level of taurine that is measured in the brain and the excitability level of the brain during the pre-seizure period.

The relationship between the "anion deficit problem," isethionic acid (ISA), taurine and brain excitability is discussed, and the experimental data are compared to test the hypothesis that taurine acts through its metabolite ISA to alter the resting potential and hence excitability in a manner predicted by the Teorell-Goldman equation. Theoretical and practical implications of this work on a new anti-epileptic agent are discussed.

CHAPTER ONE

INTRODUCTION

The discovery, isolation and identification of isethionic acid(ISA)($HOCH_2CH_2SO_3^-$) by Koechlin (1954) as the dominant component of the "anion deficit" in invertebrate nerve axons, along with the more recent identification of ISA's immediate precursor, taurine, ($^+H_3NCH_2CH_2SO_3^-$) as a normal rhythm stabilizer in mammalian cardiac tissue Read and Welty (1963) has led us (and independantly others) to predict and then establish anti-epileptic activities of taurine, Van Gelder (1972), Barbeau (1974) and Thursby and Nevis (1974). The metabolic relationship of taurine to ISA suggests to us that taurine might influence the ionic balance in mammalian neurons to supress abnormal brain electrical activity and epileptic seizures. Our prediction has been confirmed by us (and again independently by others) during the past four years. The purpose of this dissertation is to describe quantitative relationships between normal and abnormal electroencephalograms (EEG's) in experimental epilepsy and the taurine concentrations in the brain of the experimental rat.

Electrical Activity of the Nervous System

Historically a great controversy developed between two scientists of the 1700's, Nicolai Volta and Luigi Galvani, as to the origin of muscle activity in frogs' legs hung from a metallic rack. Volta insisted it was due to chemical processes as a result of the tissue electrolytes and the metal rack. Galvani explained the phenomena by the presence of an "animal electricity." In reality they were both correct and eventually their observations would lead to the inception of the field of electrophysiology and the invention of the Voltaic Pile. Their work was perhaps the first interaction between biologists and physicists and was perhaps the first application of biophysics to the understanding of the biological realm. Furthermore these "animal electricity" studies eventually led to the development of classical electromagnetic theory as developed by Faraday and Maxwell, and their applications in electrical engineering as initiated by Hertz, Heavyside and others.

This early "animal electricity" work undoubtedly influenced Bernstein in his remarkable prediction, the cytoplasmic hypothesis, where with little direct physical evidence he deduced that there existed an electrolytic solution on the inside of the cell (called the cytoplasm), which was surrounded by a membrane (called the cellular membrane). Bernstein also predicted that the conduction of the nerve impulse was due to ionic fluxes across the membrane. Both of his predicitons were much later proven to be remarkably accurate, as generally accepted by modern electrochemists and neurophysiologists. Neminski in Russia, and Caton in the United Kingdom, were probably influenced by Galvani's work which led them to the measurement of animal brain electrical activity. These measurements seem simple in light of today's technology; however, when considered in view of the fact that no means other than mechancial advantage existed for the amplification of the feeble electrical signals it seems remarkable that they were able to record the small changes at all. These measurements were indeed made with the mechanical advantage gained through the use of the string galvanometer. Bernstein's basis for the early ionic-membrane theory of bioelectricity, included the ionic theory of Arrhenius and Ostwald and the studies on "permeability" and osmosis by Overton and others.

Höeber was the first experimentalist to verify Bernstein's hypothesis by carying out several enlightening experiments. He measured the low (1 kHz) and high (10 mHz) frequency resistances of the red blood cell suspensions. In addition a suspension of red blood cells was treated with a solution to break down the membrane and the low and high frequency measurements were made again; the results showed that at low frequencies the suspension of intact cells showed a higher resistance than the haemolyzed cells and that the latter showed similar resistivity to that of the high frequency measurements. These experiments indicated that the red blood cell was covered with a thin, high resistance membrane and that indeed, as Bernstein had predicted, there existed an internal solution of low restivity (~200Ωcm).

In some very advanced measurements for the time, Fricke measured the capacity of dog blood at 87 kHz and found it to be 0.81 µf per square centimeter. Then using a dielectric constant of three he was able to estimate the thickness of the membrane to be 33 Å. This gave researchers the first usable measure of the thickness of a cell membrane, but early morphologists rejected this view. Only with the advent of electron microscopy in the 1940's and 50's was this view confirmed. (W.J. Schmidt had really confirmed these concepts using polarization microscopy in the 1930's, but this too was considered too indirect.)

These founding fathers of membrane function studies along with people like Sherrington and Raymond-Y-Cajal in neurophysiology gave the broad base necessary to initiate the study of the nervous system on both a cellular and subcellular level as well as from a system approach.

Recently, Ling (1973) and his school Goldsmith and Damadian (1974) have attempted to challenge the "membrane theory of bioelectricity," but their views are not widely accepted as they appear to omit some of the observations and valid conclusions of both earlier and later workers, such as Cole, Hodgkin and Huxley, Keynes, and Teorell. Reduced ionic mobility in dense protein solutions produced by "bound water" appears to effect this problem of the physico-chemical basis of the "animal electricity." These elements will be considered in the formulation of the present hypothesis concerning the role of organic anions in mammalian brain.

Physiology and Anatomy of the Mammalian Brain

As was indicated above, the EEG is thought to reflect changes in slow potentials (postsynaptic potentials) in cortical structures and predominantly in the apical dendrites of the pyramidal cells in layer four. In this section these structures will be considered briefly and their ability to produce electrical potentials will be discussed. Application to the mammalian brain, will be stressed, and appropriate comparison of rat brain to human brain made when necessary.

Gross Anatomy

The outer surface of the brain is covered with a blanket of cellular tissue (the cortex) 2 mm thick in rats and 6 mm thick in man. It is in this grey matter that many cell bodies of the neurons of the brain are located. Many other cell bodies are located in subcortical regions in clusters called "nuclei," such as the basal ganglia and various nuclei in the brain stem. From Figure 1-1, it can be seen that there are different cortical layers of cell bodies which neuroanatomists generally classify into six layers. The following is a brief description of the six layers of the cortex.

Layer one consists of the uppermost segments of the apical dendrites of the pyramidal cells as well as the horizontal cells and some short axon cells. This is where it is believed that the EEG, at least in part, originates, in the beginning of the apical dendrites of the pyramidal cells whose bodies lie deeper. Layer two contains the more proximal portions of the same dendrites, along with some of the pyramidal cell bodies , while layers three and four contain most of the pyramidal cell bodies. In layer four are some of the axon hillocs of the pyramidal cells along with the dendrites of the stellate cells and other interconnecting neurons and fibers. The ascending fibers travel somewhat obliquely through this layer. The next

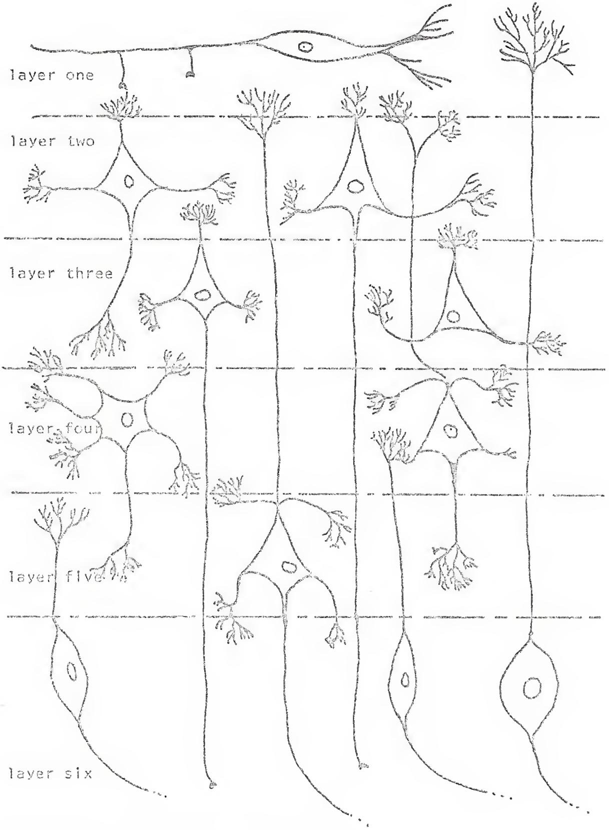

Figure 1-1 The Six Layered Cortex

level, layer five, is characterized by the deep(smaller) pyramidal cells. While layer six is predominantly made up of the spindle cells. There is some overlap and of course astrocytes and oligodendroglial cells are present in all layers. The latter are most dense in the "deeper" white matter which is almost exclusively composed of myelinated fibers. The specific afferent fibers from the thalamus impinging upon the dendrites of the stellate cells(S1-type) of layer four and in turn the axons of these cells through cartridge synapses established around apical dendrites form the connection with the pyramidal cell. Other complex communications occur involving various layers, Everett (1967), the above is a simplified but useful description of the system.

Physiology of the Nerve Cell

Neuronal anatomy

Morphologically all tissue is composed of cells. In the present discussion we are concerned with the cells of the brain. They consist of neurons and the associated glial cells. We will focus on how the cells and their chemical constituents effect the electrical waves of the brain or the " electroencephalogram."

The resting potential

Early measurements of the resting potential(and the closely related injury potential) were made by Cowan(1934) who reported that the potential between a point of injury and the normal axon membrane in a non-medullated crab nerve (*Maia*) was 30 mV. Other similar measurements ranged from 10 to more than 40 mV. The "true" resting potential was eventually measured by Curtis and Cole and also Hodgkin and Huxley who used a fine capillary microelectrode inserted into the axon and an external electrode to measure the actual trans-membrane potential. Both groups reported values around 61 mV for invertebrate fibers.

Chemical analysis of extracellular fluid and axoplasm for invertebrate fibers was carried out by Bear and Schmitt (1939) and the results are shown in Table 1-1. These data represents the first direct quantitative measure of electrolyte content for any nerve cell. This paper also provided the first definative statement of the "anion deficit problem." This work tended to verify Bernstein's hypothesis and further provided a value for calculation of the theoretical membrane potential. Later, in Schmitt's laboratory, Koechlin (1955) identified isethionic acid as the major organic anion in squid axoplasm, Table 1-2 shows his results. Using Koechlin's results with the "Teorell-Goldman equation" we can calculate a membrane potential of

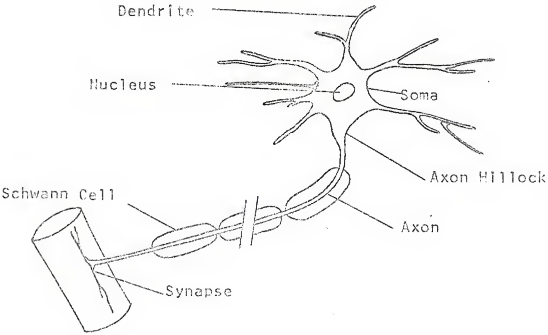

FIGURE 1-2 The Nerve Cell

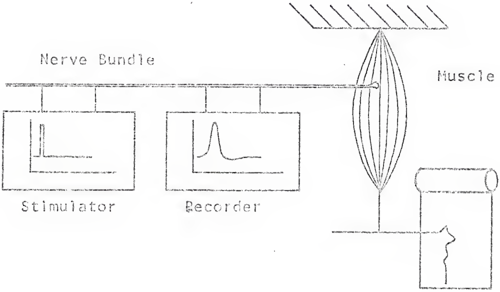

FIGURE 1-3 The Nerve Muscle Preparation

TABLE 1-1
Chemical Constituents of Squid
(Bear and Schmitt(1939))

Substance	Axoplasm	Blood	Sea Water
Total Base	600	670	540
K	310	17	12
Cl	130	530	510
Total Base-K (Chiefly Na)	310	650	530
Total Base-Cl (Anion Deficit)	490	140	30
Total Base+Cl (total Ions Analyzed)	750	1200	1050

All Values in $\frac{\text{meq}}{\text{Kgm}}$

TABLE 1-2
Ionic Constituents of Squid Axoplasm
(Koechlin (1955))

Anions		Cations	
Cl	140	K	344
Phosphate	24	Na	65
Aspartate	65	Ca	7
Glutamate	10	Mg	20
Fumarate Succinate	15	Organic Base	84
Polycarboxylate	35		
Isethionate	220		
Total	509 $\frac{\text{meq}}{\text{Kgm}}$	Total	520 $\frac{\text{meq}}{\text{Kgm}}$

the frog Figure 1-3. With this preparation an electrical stimulus is applied to a distal nerve segment and two responses are shown. First a series of electrical impulses are propagated along the axon and, secondly, a mechanical response is seen in the contraction of the muscle. This demonstrates the cause and effect relationship of the single nerve bundle muscle system. The propagation of the impulse along a fiber has been studied by Hodgkin and Huxley (1952) in their laboratories and a phenomenological description of the processes that go on in the propagation of the impulse has been set down. Also an analogy has been developed for the processes of conduction which follows a set of differential equations.

The Hodgkin and Huxley description of the propagation of the action potential begins with a resting membrane whose permeability to sodium ions increases rapidly with the proper stimulus and then decreases more slowly. At the peak of the sodium permeability a second effect begins. The potassium permeability increases and counteracts the effect of the continuing sodium current, thus returning the voltage to the resting state. this variation combines to form the action potential Figure 1-4.

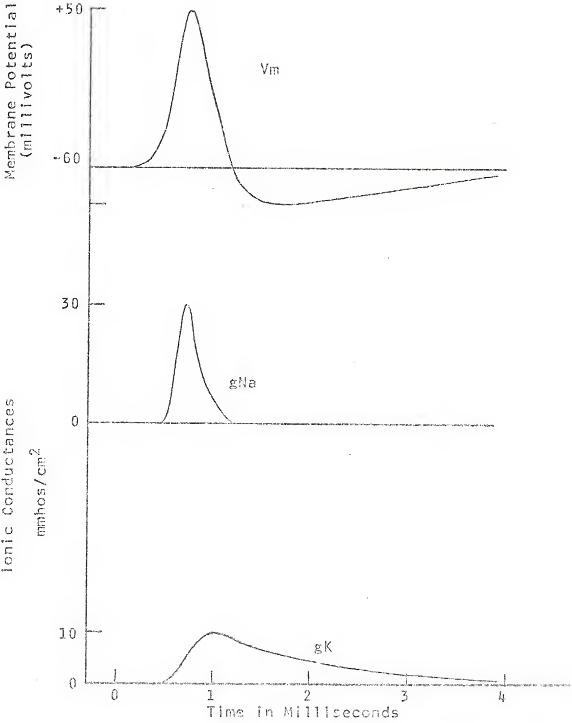

FIGURE 1-4 The Action Potential

53 mV. This value compares favorably with the measured potential found by Hodgkin and Huxley (1945) and Curtis and Cole (1942). Thus the resting potential is determined to a large extent by the concentration differences of potassium and to a lesser extent chloride and sodium across the axon membrane. In addition to the presence of inorganic ions the presence of a non-diffusible organic anion may influence the membrane potential by several methods, including the alteration of the concentration of "free" cations available for contribution to the membrane potential, provision of a non-diffusible species to affect the Gibbs-Donnan equilibrium and thus changing the Nernst potential across the membrane, or by a method of changing the permeability of the membrane.

Action potential

The axon as the "transmission line" of the nervous system must be capable of effecting changes at one end of a nerve which may be as far away as a meter from the site of the initiating stimulus (*e.g.* the motor fibers in the leg). To transmit the information along the axon a sequence of electrical impulse is generated and propagated along the axon. This phenomenon has been the focus of many studies on large single axons (from invertebrates like the squid). A good example of a nerve impulses can be seen with a nerve muscle preparation from the sciatic nerve of

The nerve cell is composed of several anatomically distinct parts: the soma or cell body is believed to house most of the biochemical processes that are involved with the survival of the neuron, the dendrite forms the primary input pathways for electrical signals impinging on the cell, the axon hillock and axon function as the initiator and transmission line respectively for the nervous impulse. This electrophysiological-anatomical system (shown in figure1-2) is the basis for communication between the cells of the brain and the motor and sensory organs. The primary site of the electrical activity of the neuron is the cell membrane as conclusively shown by many physical techniques (microelectrodes, radio-tracers,etc.). The ability of this membrane to control ion concentrations by selectively allowing passage of specific ions to one side or the other as first proposed by Bernstein (1868), and later modified by Hodgkin and Huxley (1952), is seen in both the "action potential" and the "resting potential"(nerve impulses and slow waves such as post synaptic potentials), Brock, Coombs and Eccles (1952). Many experimental data have accrued regarding the ability of the membrane to produce a potential difference across itself, and to effect certain sequential changes in the potential. To better understand this one must first consider the physico-chemical basis of the " resting potential."

Dendritic and somatic potentials

There is a fair correspondence between the action of nerve cells in the peripheral nervous system of invertebrate axon and the single nerve cell in the brain. One can at least in part extrapolate the results from invertebrate nerve fibers to the complex systems in the brain.

The summative response of the dendrites differs greatly from the all or none response of the axon. Electrically, the action potential has a duration of several milliseconds and an amplitude of 70 mV. The dendritic potentials are formed by the summation of excitatory postsynaptic potentials and inhibitory postsynaptic potentials. The characteristics of these potentials are respectivly 8 mV and 14 msec. and 25 mV and 8 msec respectively. Their electrical polarities are the opposites of each other, and arise from the chemical change in ionic permeabilities(Na or Cl respectively) induced by neurotransmitters released by the nerve impulse in a previous axon. The dendrite responds to these slow electrical potentials that are propagated electrotonically along the soma. The summation of these effects causes a hyperpolarization or partial depolarization of the somal membrane. Apparently, the extent of depolarization that occurs at the axon hillock determines whether or not the axon will fire faster or slower and thus propagate more or fewer impulse along the axon. The determining factor of whether a synapse is excitatory or inhibitory seems to rest with the type of chemical transmitter that is released at a particular site, Eccles (1952).

The formation of these potentials or change in the resting potential of the dendritic membrane is thought to be mediated by a change in permeability of the membrane to selective ions. Apparently the ability of the membrane to pass chloride ions is increased by inhibitory synaptic transmitters, whereas the excitatory transmitters increase the membrane's permeability to sodium ions. This evidence has been suggested by results of iontophoresis of the apropriate ions into the cell, Purpura et al.(1959).

It is clear that both the dendritic potentials and the axon's "impulses" are directly related to membrane structure and function, and change under various stimulating conditions (electrical, chemical or mechanical).

The EEG

Electroencephalography is a method of detecting and recording fluctuating brain electrical potentials in man and animals. It was first developed by Hans Berger (1929), although, Caton (1875) in his paper The Electric Currents of the Brain, described some "feeble currents of the brain." These were the first recorded measurements of electrical activity of the brain for any species. In these experiments Caton used rabbits and monkeys and measured the signals with a galvanometer through electrodes placed directly on the cortex. In the early 1900's Neminski was able to confirm Caton's measurements and to differentiate between two types of waves present in the brain of dogs which he named alpha and beta rhythms. Berger's great contribution was to record these signals through the intact scalp and skull of man. This work was repeatedly rejected as "impossible" until finally confirmed by Adrian (1934).

Since most nerve studies of this time had focused on the "action potential" (or nerve impulse) of single or multiple nerve fibers the neurophysiologist of the time believed that the origin of the EEG activity in the brain was the all-or-none (nerve impulse) response of the axon. Forbes (1936) was the first to point out that the slow waves observed in the cortical recordings were different from the all-or-none potentials of the axon. Renshaw, Forbes and Drury (1938), using microelectrodes, were able to record the slowly varying potentials of the dendrites of the neurons and show that the EEG was not the summation of a series of all-or-none responses, thus laying the foundation for the theory of EEG generation usually accepted today. There is strong evidence that the coherent sources of currents in the brain necessary to produce the large signals of the EEG (as compared to the feeble currents in a single cell) arise from the grouping of parallel pyrmidal cells of the cortex. It is the apical dendrites of these cells that extend up into the second and first layers of the cortex and provide the necessary homogeneity for the production of the potentials that are measured at the scalp. These are depicted in Figures 1-5 and 1-1, illustrating the pyramidal cells and the six layered cortex.

It was thought that these cells by themselves could not be entirely responsible for the rhythmicity of the EEG, and further work was done in search of a synchronizing center of the brain electrical activity. Adrian (1941) used electrodes implanted in the thalamus to demonstrate that there was an inherent rhythmicity associated with this structure. Adrian showed that with stimulation of sensory fibers the associated areas of the thalamus would respond with a rhythm that was then propagated to the cortex and

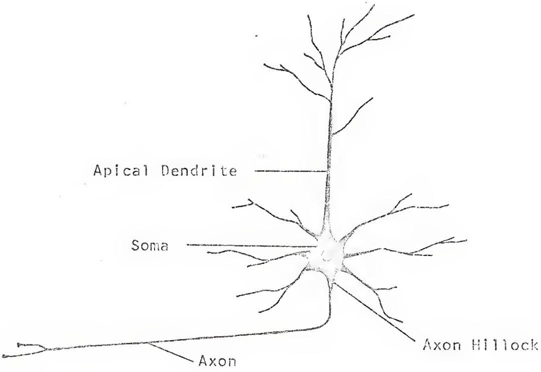

FIGURE 1-5 The Pyramidal Cell

could be recorded in both the thalamus and the sensory cortex.

Dempsey and Morrison (1943) demonstrated the dependance of the cortex on the thalamus by using direct stimulation of the thalamus, thus extending Adrian's work. Magoun (1944) in his work on the descending reticular activating system demonstrated that this complex structure in the center of the brainstem exerts a marked influence over reflex action in the spinal cord. Shortly after this Moruzzi and Magoun (1949) demonstrated the influence exerted on the state of consciousness by another related structure, the ascending reticular activating system.

Still further advancements included the discovery of the potential shifts due to hyperpolarization or depolarization of the postsynaptic membrane in the total cortical response as seen in the EEG. Thus there is evidence that the inherent rhythmicity recorded in the EEG is closely associated with these hyperpolarizations and depolarizations of the dendrites, Brock, Coombs and Eccles (1952).

Normal rat electrical activity

Phenomenalogically the rat EEG is very similar to the human EEG. There are periods of"desynchronized" electrical activity relating to what appears to be the alert state. The rat's alpha waves do appear to be shifted to a lower frequency; six to thirteen waves per second rather than eight to thirteen waves per second with the majority of the activity about seven waves per second. Delta (one half to three and one half waves per second) waveforms are similar in human and rat recordings. Beta activity (thirteen to fifty waves per second) seems accentuated in the rat cortical recordings.

The amplitude of electrical signals for the extradural recordings ranged from ten to one hundred microvolts. The variation is presumably due to "contact variation" of the electrodes with the dura and thus the brain, as well as normal physiological state variations, and variations between individuals.(These recordings are of course possible only because the large electromyographic potential normally occuring in animal recordings has been circumvented by our use of skull penetrating extradural electrodes.)

Abnormal rat electrical activity

The abnormal activity observed from rat extradural electrodes is very similar to the activity observed from scalp recordings taken from human subjects. Single spike waveforms with durations of less than 85 msec and amplitudes of 50 to 200 μV appear in rat abnormal recordings as well as in human recordings, bursts of these or similar waveforms ranging from two spikes to a train of spikes that can last for one second have been observed. Spike and wave activity of two to four waves per second has been observed in the rat. Large amplitude delta waves occur both in humans as well as in the experimental rat. Burst supression occurs, but is fairly rare in its true form; the closest form that is observed in rats is at the end of a seizure(osmotically induced) when the animals EEG usually goes through a period of isoelectric activity interspersed with some minimal electrical activity. Burst suppression similar to that described by Fischer-williams (1963) and Ellington (1968), has been seen only rarely in rats, usually after a severe seizure. This is not unlike burst supression seen in humans (i.e. this usually occurs just ante-mortem).

The abnormal behavior seen in the animals includes myoclonic jerk, mild focal automatisms, Jacksonian march, generalized seizure activity including tonic followed by tonic-clonic movements with subsequent isoelectric activity and physical exhaustion, and, finally, the very dramatic Grand Mal seizure.

Epilepsy and a Useful Model in Rats

Human epilepsy is probably as old as man himself and epileptic-like fits in animals even older. The term was introduced by the Greeks to describe the phenomenon in which a person was apparently seized by forces from the outside. Epilepsy was recognised as a disease as early as 2080 B.C. when laws and rules regarding marriage of an epileptic person and the validity of their court testimony was set down in the code of Hammurabi. Hippocrates (460-357B.C.) considered epilepsy a disease of the brain. Charles le Pois (1463-1636) first clearly stated that all epilepsies are of brain origin. Jackson contributed to the understanding of epilepsy by introducing the concept of the discharging focus in 1870, and considering Petit Mal and Grand Mal seizures to be manifestations of paroxysmal discharges beginning at a focus and spreading to more numerous areas. Gower classified the epilepsies into two categories those seizures due to demonstratably damaged focal areas and those that cannot be related to a structural change in the brain, Schmidt and Wilder (1968).

The work by Caton and Neminsky and , of course, Berger provided greater understanding of the phenomenology of the epilepsies. The use of EEG's in clinical practice has allowed for an early confirmation of the diagnosis of epilepsy in a great number of cases.

Epilepsy itself is an interesting disease manifestation because it apparently represents either local or wide spread electrical storms on the cortex of the brain. These storms are evidenced by the dynamic changes in the electrical activity measurable on the scalp or on the cortex. The use of experimental animals for the investigation of the "epilepsies" requires several constraints: comparability with humans, size of animal, cost and labor requirements among others. To be useful the animals' behavior in a physical as well as electrographic sense must be close to the system that one is attempting to model (human epilepsy). A comparison is illustrated in Figure 1-6. The size of the animal should allow for easy handling and simple surgical procedures. The cost in terms of the human effort and the specific cost of animals should be weighed against information to be gained from the experiments and with comparability of the data with those from humans. The similarity of the rat and human EEG have been compared in the figure above. Rats also provide an inexpensive, small and easy-to-handle specimen on which to preform experiments. All of these requirements are met by the rat when properly treated as an experimental model for epilepsy. The methods of inducing epilepsy may include various scarring agents such as penicillin or cobalt powder applied directly to the brain or osmotic agents introduced intraperitoneally.

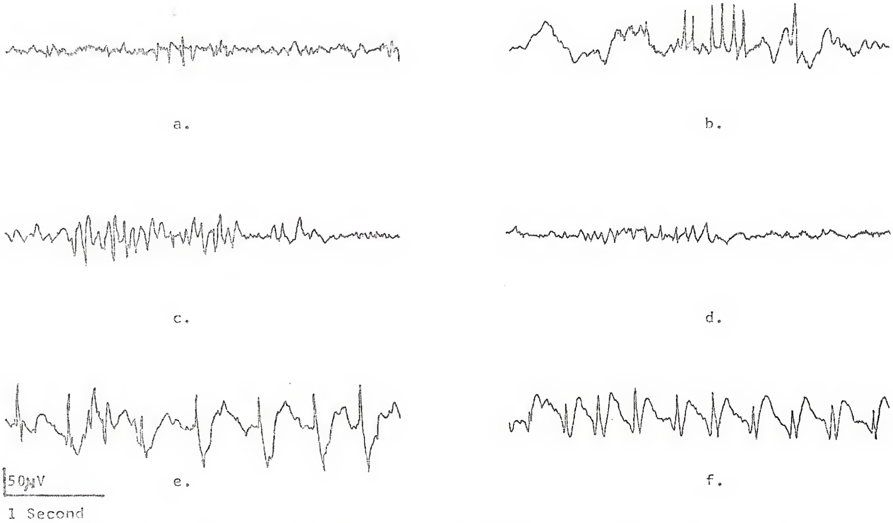

Figure 1-6 The Rat versus the Human EEG: a. Rat Spikes, b. Human Spikes, C. Rat Paroxysmal Bursts, d. Human Paroxysmal Bursts, e. Rat Spike and Wave Activity f. Human Spike and Wave Activity.

How Heart Relates to Brain

The relationship of brain to heart may seem to be out of place in a discourse on the bioengineering and biophysical aspects of taurine in the brain. However, as will be seen later, the physiology of taurine's action may be similar in heart and brain and may be modeled in a similar way. This is really not surprising though, since both are " excitable tissue," and the excitability of all animal (and plant) cells seem to have a common ionic basis.

The heart consists of specialized muscle cells connected together at the "intercalated discs." The cells of the heart are of three basic types: nodal cells, Purkinje cells and contractile muscle cells. The electrical activity of the tissue is slower, synchronous and thus slightly different from that of the more complex nervous tissue as shown in Figure 1-7. However, it is believed that the electrogenisis of the cardiac cell potentials is quite similar to that of the nerve cell. Thus, the resting potential is established by the potassium steady state flux through the cell membrane. The action potential in the heart is indeed begun with an influx of sodium ions followed by an efflux of potassium ions which is in close agreement with the action in nerve. However, the time course of events varies in that the velocity of propagation of the ionic conduction process along one cell

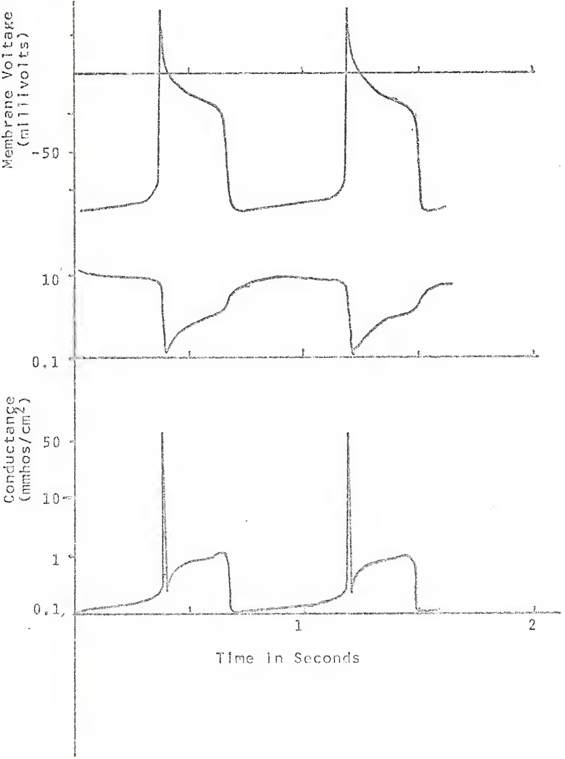

Figure 1-7 The Membrane Potential of a Purkinje Cell (Noble(1962))

to the next one is much slower in heart. As in the brain but in a much more defined pattern, the heart also has a pacemaker action. The cells of the sino-atrial (SA) node and the atrio-ventricular (AV) node perform this timing control or pacemaker function in the heart. The SA node paces the atrial muscle cells and through the Purkinje bundle cells, the AV node. The later in turn paces the firing of the ventricular muscle cells; therefore, cardiac contractions are uniformly synchronized for optimal output. The heart has only two pacemakers (connected in series) and thus the rhythm is relatively simple; in the brain there are indeed many driving centers and thus the rhythms are much more complex. In cardiac tissue if normal activity is interrupted by exogenous electrical, chemical or mechanical stimuli, the heart can stop beating, or it can beat exceedingly irregularly (fibrillation) or a regularly irregular electrical storm can be propagated repetitively over the surface (various dysrhythmias). In a similar manner the brain can be caused to exhibit quite abnormal electrical activity by similar exogenous physico-chemical stimuli. When the irregularity develops from some internal abnormality(lack of oxygen, unusual scarring, abnormal metabolism, etc.) the endogenous dysrhythmia is usually indistinguishable from an exogenously induced dysrhythmia. Indeed an exogenous agent may be useful in supressing or reversing the abnormality in the rhythm of the otherwise normal tissue Davson(1964).

The use of brain drugs in heart and visa versa

Perhaps the most well known of the anti-dysrhythmic drugs used in both brain and heart is diphenyl-hydantoin (DPH). The use of DPH in heart abnormalities has been sucessfull in treating premature ventricular contractions, paroxysmal atrial tachycardia, paroxysmal atrial fibrillation and many others. Many other cardiac drugs do not greatly affect the brain. Apparently they do not enter the brain as freely as does DPH. The use of DPH in the central nervous system includes treatment of epilepsy, thought-mood and behavior disorders, stuttering, psychosis and other maladies, Bogoch (1970).

The first application of taurine for theraputic use was in reducing the cardiac dysrhythmias in dogs. The effect of taurine on digtoxin induce dysrhythmias was dramatically demonstrated by Read and Welty (1963). In their work taurine was administered both before and after the injection of digitoxin, and in both cases either the arrythmias never appeared or stopped. This work led to our independant speculation (about 1970) that like DPH perhaps taurine might show some effects in the brain. With subsequent independant confirmation by Van Gelder(1972), Thursby and Nevis(1974), Barbeau(1974).

CHAPTER TWO

HISTORY OF THE "ANION DEFICIT PROBLEM" AND THE ROLE OF TAURINE IN CONTROL OF BIOELECTRIC POTENTIALS

The Anion Deficit Problem

The apparent anionic imbalance in nervous tissue was first described in the work done on the ionic concentration of single nerve fibers by Fenn _et al._ (1934) who cited the study of the ionic constituents of the nerve, Cowan (1934). That is that when one considers the constraints of charge neutrality in in an electrolytic solution(the cytoplasm or extracellular fluid) as well as the necessity of equlibrium of osmotic pressures from the inside to the outside of the membrane it was found that there was a deficiency of anionic species within the axon. Fenn _et al._ reported (among other observations) that "there appears to be a large excess of inorganic cations over anions in the potassium space"(axoplasm).

However, in 1939, Bear and Schmitt specifically formulated the "anion deficit problem" in studies on squid axoplasm. They realized that these deficient anions must be of great importance in the biochemistry of the axon;

they also noted that the sum of all known ions at that time could not account for the deficit. From the physical evidence given they predicted that the anion was organic in nature and had a molecular weight of about 100. This paper was the first formal statement of the axoplasmic "anion deficit problem." The anion deficit was thought to range from 25 percent to 40 percent of the total internal ionic concentration; later this was shown to be an underestimation.

Silber and Schmitt (1940) initially appeared to be able to explain the majority of the deficit by amino acid constituents leaving 0.06 mgm per gram of water in the anion deficit (i.e. only 12 percent residue of ICF). This suggestion had two points of difficulty; first, if these amino acids had been responsible for the deficit they would have had to be dicarboxylic in nature in order to contribute to the anion pool; secondly, the authors failed to detect the presence of sulphur in their preparation. Indeed, they were looking at amino acids in such a manner that the true anion was probably removed in one of their extraction steps. In their paper they stated that the amino acid concentration did not change for significantly different external conditions: anoxia, hyperactivity and thermal changes. This however would not preclude changes in the structure of the associated groups (i.e., the conversion of cysteine to taurine to isethionic acid) even had they then identified ISA as subsequently done by Koechlin in Schmitt's laboratory.

Silber in his dissertation (1941), described the amino acid content of lobster nerve. He states " less than one third of the anion deficit appears to be accounted for by the available acid group of aspartic acid with less than half of the remaining deficit made up by Cephalin and keto-acids." Allowing for this anionic contribution, he reports the anion deficit to be 0.205 mEq per gram of water, or approximately 40 percent of the total anions.

Schmitt Bear and Silber (1939) described the anion deficit in lobster nerve in even greater detail. In lobster nerve, a deficit of 0.33 mEq per gram of water was found. Further, titration curves showed that the anion present was in the form of an acid, and from earlier conclusions it was described as an organic anion. The level of non-protein nitrogen was such that they further concluded that the acid was probably an amino acid. The major breakthrough in the anion deficit problem came when Koechlin (1953) began to demonstrate the anionic species available in squid nerve axoplasm. With his improved techniques Koechlin was able to extract the major anion and identify it as isethionic acid (ISA). Koechlin (1955) presented a further detailed analysis of the anion deficit.

With his second paper the ionic balance sheet was complete within 11 μeqv per gram of axoplasm. In a total of 520 μeqv per gram only two percent remained un-identified. Koechlin pointed out that this was the first reported occurence of ISA in any biological tissue. He speculated that this was the metabolic product of taurine which was also found in the axoplasm. Of the chemical species present in axoplasm Koechlin reported that the most abundant was potassium, followed by isethionate, then chloride and the other ions. His paper not only resolved the anion deficit problem in squid but it posed many interesting questions as to the general biological significance of ISA and its precursor taurine.

Deffner and Hafter in a series of papers (1959, 1960a, 1960b,1961a, 1961b) investigated in detail the chemical composition of squid axoplasm. Along with discovering several previously unnoticed compounds they confirmed Koechlin's measurements on the anionic content of the fluid. All of the work by Deffner and Hafter and also that by Koechlin was strongly motivated and guided by the director of the laboratory, Professor F.O. Schmitt.

Challenger (1970) has suggested that the presence of isethionic acid in biological tissue may be involved in polysaacharides, mucopolysaacharides and lipid function. He sites as a basis for this concept the fact that an ethyl-phosphonic acid(i.e. a phosphorous analogue of ISA) is found in rumen protozoa by hydrolysis of the lipid extract.

Hoskin and Brande (1973) discuss a new method for the determination of ISA in biological tissue. The method of enzymological assay is old but only now has been applied to the assay for ISA. Their work confirms Deffner and Hafter's work on the level fo ISA in squid axoplasm. The method of differential enzyme activity to determine the ISA concentration may be useful in other tissue in the future.

Jacobsen and Smith (1968) provided an exhaustive review of the litterature pertaining to the biochemistry and physiology of taurine and its derivatives prior to 1968. In the following paragraphs there will be a brief discussion of the literature that has been written since the work on taurine and ISA by Jacobsen and Smith.

Reddy (1970) using tracer studies evaluated the active uptake of C-14-taurine against concentration gradients in rabbit lens. He found the process to be inhibited by ouabain and by an absence of calcium. This links the activity of taurine with Na-K-ATPase. He also noted that if the *in-vivo* potassium level is decreased, the rate of uptake of taurine is reduced. The transport of taurine is inhibited by glycine, alanine and arginine presumably by competition between taurine and these amino acids.

Naito etal. (1972) have tested the effects of a derivative of taurine, taurinopiperidine, on several laboratory animals. They concluded that taurinopiperidine has no harmful effects on the animals at high dosages and that it would be suitable for further pharmacological testing. The piperidine moiety may provide for ease of passage of the total group across the blood brain barrier.

Sorensen (1973) measured the levels of twenty amino acids including taurine in excitatory, inhibitory and sensory fibers of the claw of the crab, Cancer magister. She reports that the only significant difference found was in GABA levels. The predominance of GABA in the fibers was in the ratio of 46:3,inhibitory: excitatory. The high and approximately equivalent level of taurine in inhibitory and excitatory fibers may be of some significance in that again taurine's ubiquitous nature seems to indicate a more universal role in the function of the nerve cell.

Occurence of Taurine in Mammalian Brain

Jasper and Koyama (1969) described large increases of the release of taurine from surface cortical neurons under several different conditions including arousal, stimulation of the reticular formation and the thalamic bodies. The EEG waveforms described for reticular formation and thalamic stimulation appear to be highly abnormal(e.g., 3-4 per second spike and wave activity). Battistin, Grynbaum and Lajtha (1969) reported the distribution of taurine in various regions of the cat brain. Taurine had the highest concentration in the corpus callosum and was lowest in the thalamus. However slices from the thalamus showed the largest uptake of taurine on incubation. Johnston (1969) showed the distribution of taurine in cat spinal cord.

Guidotti, Badiani and Pepeu (1972) have investigated the occurence of taurine in cat brain with respect to anatomical structures. They have also investigated the change in the concentration of taurine with transection at mid-pontine and collicular levels. The concentration of taurine found for different neurological states was slightly different, however not statistically significant.

Perry _et al._, (1971a) analyzed the amino acid content of twelve different regions of human brain at autopsy and taurine was found to be highest in concentration in the cerebellum. Perry _et al._ (1971b) analyzed the amino acid content of human biopsied and autopsied brain samples. Taurine did not show a significant change in the level between biopsy and autopsy. Of significance is the high level of taurine in human brain and animal brain. Perry _et al._ (1972) measured the concentration of 32 amino acids in five areas of cat brain and showed taurine to be highest in cerebellar tissue. Taurine was found to be the third highest overall in concentration in cat brain.

Perry and Hansen (1973) described the presence of hypotaurine in rat brain. They also indicated that the taurine level did not change significantly after decapitation prior to freezing (two hours); however, they suggested that since GABA and other compounds did change rapidly a quick freezing technique was preferable to allowing the tissue to remain at room temperature for extended periods of time.

The level of taurine in brain slices

Davison and Kaczmarek (1971) have shown a release of taurine from brain slices upon stimulation that is similar to the release of gamma-amino-butyric acid (GABA). They used this evidence to enhance their hypothesis that taurine is possibly a neurotransmitter in the central nervous system. Kaczmarek and Davison (1971) described their initial experiments in which they stimulated radio active taurine loaded brain slices and observed the concomitant eflux of taurine. They also stated that taurine has been reported to have been absorbed from the brain slice media. They stated that their results enhanced the possibility that taurine is a neurotransmitter.

Oja (1971) described the exchange of taurine in adult and newborn rat brain slices. The author showed an active uptake of taurine into brain slices. He also indicated a similar high level in the plasma of new born animals. He indicated that the taurine flux did not reach equlibrium state with either high or low taurine concentrations in the external medium.

Kaczmarek and Davison (1972) studied the efflux of taurine from brain slices during electrical stimulation. They found that taurine was released from the brain slices upon stimulation and that replacement of calcium by magnesium in the medium decreased the eflux of GABA to a much greater extent. The authors suggested that taurine may indeed be a neurotransmitter and that the hypothesis should be tested in-vivo. They also suggested that a search should be made for synaptic vesicles where taurine is the major transmitter present.

Lahdesmaki and Oja (1972) attempted to characterize the transport mechanism of taurine into brain slices. They showed that the affinity of taurine for the transport system is higher in new born animals; however the maximum velocity is lower than in adults. In adults electrical stimulation increased the maximum velocity but did not influence the affinity of taurine for the transport system.

Crnic, Hammerstad and Cutler (1973) studied the efflux of taurine from brain slices into media containing different amino acids. The rate of release of taurine from brain slices was increased with solutions containing beta-alanine, GABA and taurine.

Lahdesmaki and Oja (1973) compared the active transport of compounds that are structuraly similar to taurine into brain slices. Compounds that inhibit the uptake of taurine include hypotaurine, GABA, N-methyl taurine and l-cysteic acid. The compounds that were found to be non-inhibitory were: ISA, l-methionine sulfoxide, l-cysteine, glycine, amido-sulfonic acid and l-cysteine sulfinic acid. The authors recognized two features of the list and the transport system. First "the hypothetical carrier" of taurine at brain cell membranes "recognizes" in the acceptable molecule both strongly ionized electronegative and electropositive ends, supported by at least two carbon atoms. Second, "two is the optimal length of the carbon chain in such a molecule." However the authors fail to recognize that all of the inhibitory species are of sufficient length and charge distribution to form a cyclic structure (internally compensated zwitterion). The non-inhibitory compounds lack the length or amphoteric character.

Metabolism of Taurine

Gilles and Schoffeniels (1968a) described the uptake of C-14 from NaHCO3 into taurine molecules, as well as aspartic acid and glutamic acid. They postulated that there must be an unknown pathway that would allow incorporation of C-14 from NaHCO3 into the taurine molecule. In their second paper (1968b) they refined their approach to include the hypothesis that apparently the metabolism of taurine is based on the presence of pyruvate.

Kaczmarek, Agrawal and Davison (1970) studied the presence of taurine and its probable forming enzyme, cysteinesulfinate decarboxylase, and showed that there was a steady increase in the enzyme activity with time in a parallel manner to the increase in taurine. Also taurine and the accompanying enzyme have been isolated in mitochondrial and synaptosomal tissue. Agrawal, Davison and Kaczmarek (1971) demonstrated a decrease in taurine level with a decrease in cysteinesulfinate decarboxylase activity.

Huxtable and Bressler (1972) have studied in detail the distribution and metabolism of taurine and ISA in rats.

The most significant findings were in the measurement of the uptake rate of ISA in brain. There is a time period of seventy hours required for maximum absorption of the anion in brain as well as in all other tissue. As did Awapara (1957), the authors described the peak uptake of taurine as requiring about four days.

Valcana, Hudson and Timiris (1972) showed small and insignificant changes in the level of taurine and other compounds between normal and x-irradiated post natal cerebellar tissue.

Sgaragli and Pavan (1973) were unable to show any metabolic change due to intraventricular injection of neutral amino acids including taurine.

Central Nervous System Changes in Taurine Level

Enwonwu (1973) showed no variation of the level of taurine in the brains of rats with ascorbic acid deficiency.

Fowler (1973) showed an elevated GABA level in rat brains after the administration of Ethanolamine-O-Sulfate. The same study showed no significant change in the level of taurine during the same treatement.

Taurine and Convulsions

Van Gelder (1972) described the antagonistic action of taurine in cobalt induced epilepsy in cats and mice. His data included a demonstration of the reduction in the number of seizures caused by the topical application of cobalt powder on the surface of the brain. In addition he reported that the level of taurine was reduced in the brains of cats and mice subjected to convulsions from cobalt powder applications. He suggested the possible theraputic use of taurine in treatement of epilepsy and the urgent need for further investigation.

Thursby and Nevis (1974) studied the effects of taurine on the nervous system of rats and mice. Using electro-shock testing(EST) it was shown that taurine offered protection against seizures caused by EST. In addition, using rats they have shown that the proclivity of having seizures when subjected to osmotic stressing was reduced in animals treated with taurine.

Van Gelder, Sherwin and Rasmussen (1972) studied the concentration of some amino acids in human brain biopsy samples from patients with epilepsy. Their data showed that apparently the amino acid concentration in the tissue at the focus is slightly lower than at a site peripheral to the abnormal site. However, it appears that the necessary controls in normal patients are understandably missing. In addition to the brain tissue studies, total blood concentrations of taurine would be helpful to determine the relative level of taurine in the brain compared to the blood in the patient.

Izumi et al. (1973) administered taurine to rats intraventricularly to reduce the effects of ouabain induced seizures. Their results showed that taurine could significantly reduce the strength and incidence of seizure activity.

Haas and Hosli (1973) have studied the interaction of taurine with two convulsants bicuculline and strychnine on the firing rates of brainstem neurons. They have placed taurine in the category of a " glycine like" amino acid transmitter. They point out that the apparent "taurine receptor " is similar in structure to the "glycine receptor." They propose as others have that taurine should be considered as an inhibitory neurotransmitter. King, Carl and Lao (1974) showed about ten percent change in glutamic acid , glutamine and aspartic acid with larger changes in alanine and GABA concentrations (twenty and thirty percent) during and immediately after seizure activity. These results on mice indicate that there may be some amino acids whose concentrations change during seizures.

Barbeau and Donaldson (1974) showed that taurine was effective in decreasing ouabain induced seizures in rats.

Urquhart _et al._ (1974) described three experiments in which they determined that taurine was absorbed from the gut of rats into the brain, and that when taurine was administered parentrally in monkeys it was also absorbed into the brain. In addition they showed that when taurine was administered oraly to human subjects the plasma level of the compound was increased significantly. They also showed no side effects in the human subjects. They concluded that the "oral administration of taurine might prove effective in correcting a taurine defficiency in human brain when this occurs."

CHAPTER THREE

RATIONALE

The ubiquitous presence of taurine in nature is evidenced by the abundance of literature reporting the occurence of taurine in almost every genus and species in the animal kingdom and in many forms of plant life. Its function in tissue has been inferred and attempts have been made to hypothesize a reason or list of reasons for its occurence and abundance in nature. Various roles from that of an energetic metabolite to that of an osmotic regulator have been proposed. Whether taurine fulfills one or more of these roles among a multitude has not been demonstrated. It is believed by some investigators that the role of taurine is in effect a combination of roles. Taurine may appear to act as a binding agent, osmo-regulator, membrane voltage stabilizer and especially a balancing anion. This can be understood in terms of a Teorell model-like interaction, in which taurine would contribute to the transmembrane ionic balance and therefore to the membrane potential, as well as to the osmotic balance. Within all these roles is embodied the concept of a stabilizing element for the membrane potential and, hence, for any cellular aggregrate whether it be muscle, ganglia or brain. Taurine's ability to "quiet" abnormal electrical activity in the heart muscle (as induced by digitalis-like compounds) is ample proof of this stabilizing overall action in a collection of excitable muscle cells through stabilization of the membrane potential. An extension of this concept leads one to the idea that taurine may exert a similar force on any organized group of nerve cells. Such a system is found in the various centers (cortical or subcortical or lower) of the brain. This hypothesis has been supported by a few researchers in several different laboratories. Their interests are usually limited to rather narrower aspects of neurophysiology, or neuropathology. Even if the empirical observations of taurine as a membrane function stabilizer were widely recognized, the mode of action of taurine as both an electric potential, and osmotic regulator remains to be fully understood. Hence, the questions of the effect taurine has on the membrane and how it affects an inhibiting or quieting response are very important ones.

Inorder to better understand the brain and its functions, one must evaluate its measurable parameters or "outputs." These include such things as thought processes, motor responses to stimuli, and certain physical parameters, for example the EEG or brain pressure or chemical constituents. One can put different types of inputs into the system by several different pathways: the input may be visual, auditory, chemical , osmotic, or electrical in nature. The output may be recorded by the measurement of the electrical potential on the surface of the brain (or externally), by the pressure of the cerebrospinal fluid, or by the changes in chemical concentration found in the brain of certain compounds. The total picture of how the brain functions, depends upon many factors, and thus, to elucidate brain function one must study multiple parameters rather than just one or two.

The presence of taurine, one of the major amino acids, in excitable tissue, especially in the central nervous system, leads one to question why such high concentrations of this compound have been placed in the brain by evolution. The apparent stabilizing effect that has been demonstrated in several different species of mammalian brain and in different cellular preparations in invertebrates leads one to wonder what the actual stabilizing action that this compound exerts on the membrane system. There are several different approaches that one could take in trying to understand taurine's effect on the brain. Here we take the approach that would apply our previous work done in this laboratory, namely a quantitative assay of the brain in normal and hyperexcitable states, i.e. the experimental epilepsies. This would seem to give the most definitive information on the action of this compound on the stability of the membrane electrical activity.

The use of epilepsy as a model to test the anticonvulsant effects of a drug are well documented. The analysis of the EEG to determine the effects of drugs on the central nervous system is now well accepted, Goodman and Gilman (1970). In this work we have used a new method of quantitatively analyzing the abnormal EEG with the reproducable induction of seizures by osmotic imbalance. Thus one can test the in-Vivo changes of the endogenous levels of taurine in the brain during controlled and thus reproducible and quantitative states of hyperexcitability. It is expected that with a better knowledge of the way that the amount of this compound varies during the course of a seizure, that insight as to how the compound may influence the excitability of the brain will become evident. With this type of information it should be posible not only to evaluate the present models for the membrane but also any model of epilepsy, whether at the cellular level or at the system level. Furthermore, one should then be able to deduce the means by which taurine exerts its controlling (inhibitory) action. As in a "black box" or systems analysis one can deduce a series of possible equations that should characterize the black box (system) input-output relationships. However, without a knowledge of the internal details of the system, one can never really understand the actual operation of the system. This is especially true of the brain and, therefore, it is truly essential to bioengineering to understand the functional basis and detailed mechanisms of the nervous system. Thus eventually the better understanding of the function of the brain depends upon knowing its major constituents and their roles in the total system. The use of chemical as well as physical techniques to gain a better understanding of this system is not merely justified but essential.

Detailed Rationale

The EEG, in as much as it reflects the electrical activity of the brain, is a measure of the electrical activity of the neurons and ultimately the cell membranes. It is a reflection of summated potentials originating in the nerve cell membrane. Thus in a gross manner the measurement of the EEG is a measurement of the electrical activity of the cell membrane. Since taurine has been shown to affect the EEG and the behavioral response of animals, it seems valid to question the role played by this anionic species in the electrogenesis of the nerve membrane potential and in the generation of the EEG.

In beginning to answer the question of why taurine is present in the nervous system of so many animals, one is confronted with the following three considerations. First, taurine is one of the most concentrated compounds in the nervous system of most species. Where it is not in such high concentrations its metabolic product ISA, and in a few cases glycine, are in compensatingly high concentration. Secondly, in reviewing others' work on heart, one observes a relation between taurine concentration and abnormal versus normal activity (dysrhythmias) of the heart. Taurine has been suggested as a cardiac antiarrythmic for use in animals and humans. Most recently other laboratories as well as our own (Van Gelder (1972) and Thursby and Nevis (1974)), have suggested that taurine may be of use in the control of seizures in animals and humans. The evidence presented points to the hypothesis that taurine has a stabilizing effect on the excitable membrane whether it is in the heart or the brain or possibly other tissue such as voluntary muscle. Thirdly, the normal body level of taurine must in some manner control the stability of the nervous membrane and consequently the electrical activity of the brain as a system. For example, circulating potassium ion levels play a key role in many tissues, especially in heart. Taurine may act to prevent a sudden massive release of potassium from the large muscle masses. (This may be especially significant in certain myopathies).

From this basic point it is seen that taurine must have some fundamental role, and such a role should be relatable to various parameters through specific biophysical principles of known membrane potential generation Nernst (1908), Hodgkin and Huxley (1952), and Teorell (1962). Such a relationship may possibly be explained by the following hypothesis.

The presence of taurine as a free constituent of nerve fibers and the central nervous system, as well as its ability to control the dynamic fluctuations in the nervous system leads one to speculate as to the reason for this control. The relationship of ISA to the anion deficit is clear. However taurine's function as an anion or as the precursor to an anion has not been proven conclusively. Assuming for a moment that taurine could act as an anion (by masking the amine group), let us look at the effect this anionic concentration might have on the membrane resting potential and the action potential. A complete discussion of the electrical phenomena occuring at the membrane is contained in Teorell (1953). Consider the Teorell-Goldman constant field eqution;

$$Vm= \frac{RT}{nF} \ln \frac{pk\,[K]^{o}+pNa\,[Na]^{o}+pCl\,[Cl]^{i}+pA\,[A]^{i}}{pk\,[K]^{i}+pNa\,[Na]^{i}+pCl\,[Cl]^{o}+pA\,[A]^{o}} \quad . \qquad (1)$$

From first considerations one would omit the effect of the free anionic species A-. However the sodium, potassium and chloride concentrations are affected by the non-diffusible anion II. If we assume that the anionic concentration on the inside is much greater than the same species' concentration on the outside and that the permeability(pA-) is almost zero, then we can write from charge neutrality considerations;

$$[Na]^i + [K]^i = [Cl]^i + [A]^i \quad \text{(Inside)}$$
$$[Na]^o + [K]^o = [Cl]^o + [A]^o \quad \text{(Outside)} \qquad \text{(II)}$$

From Table 3-1;

$$[Na]^i, [Cl]^i << [K]^i, [A]^i \quad \text{(Inside)}$$
$$[A]^o << [Na]^o, [Cl]^o, [K]^o \quad \text{(Outside)}$$

Thus;

$$[K]^i \cong [A]^i$$

Therefore I Becomes;

$$Vm = \frac{RT}{nF} \ln \frac{[K]^o + p1[Na]^o + p2[Cl]^i}{[A]^i + p1[Na]^i + p2[Cl]^o} \qquad \text{(III)}$$

From III one could expect that the anionic concentration on the inside of the membrane would have a strong effect on the resting potential of the membrane and similarly it would exert an effect on the concentration of the other

TABLE 3-1
Constituents of Mammalian Brain
(Schmidt and Wilder (1968))

Extracellular		Intracellular	
Na	140	K	146
K	5	Na	13
Cl	130	Cl	8
Mg,Ca etc.	15	Nondifusable Anions	120

All values in $\frac{\text{meq}}{\text{l}}$

ionic species in the axon or cell. As has been mentioned earlier, it is not known how taurine exerts a control over the excitability of the membrane. However there are several actions which one could propose. First, by masking the amine group taurine itself would become an anion and thus have an effect on the potential directly. Second, that the metabolism of taurine to ISA although slow, would provide the anion for the system. Thirdly taurine could participate as a fixed charge on the membrane surface thus affecting the total ionic character of the membrane.

CHAPTER FOUR

EXPERIMENTAL PROCEDURES

This chapter is designed to describe in detail the methods of experimentation that have been used to obtain the data to be presented in the next chapter. It has been the author's purpose to provide a set of data that will allow some additional insight into the function of the central nervous system with particular emphasis on the role of taurine in the abnormal electrical activity of the brain of rats. The procedures were designed to give data on the level of taurine in the brain at varying times during the electrographic and behavioral seizure activity. The correlation of electrical and biochemical activity is essential to an understanding of the interrelationship that occurs between them.

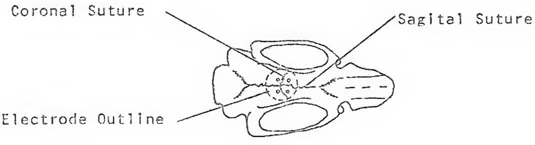

Figure 4-1 Implantation Scheme

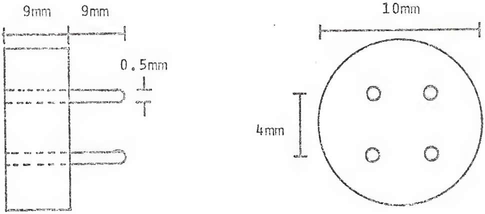

Figure 4-2 Electrode Assembly

Animal Procedures

Male albino rats of the Sprague-Dawley strain were used exclusively in these experiments. In general the animals' weights were maintained between 195 gm and 275 gm at the time of experimentation. The animals were implanted with a four element electrode array, Figure 4-1, at a point centered on the bregma. Details of the implantation procedure from Thursby (1971) and Thursby and Nevis (1973) follow. The present array of electrodes consists of a modified Augat No. 8058 operational amplifier socket with pins of gold-plated brass. The socket is modified by removing alternate pins to provide a square array of four electrodes four millimeters apart, Figure 4-2. The pin electrode assembly was implanted extradurally, through four burr holes in the skull, each placed symmetrically two millimeters from the coronal and sagital sutures. Avoidance of any cortical injury is imperative. The electrode assembly is mounted in place by two anchor screws(stainless steel, size 000) placed in the bone and covered with cranioplastic cement, Figure 4-3. The animals were allowed to recouperate, post-operatively for a one week period prior to experimentation.

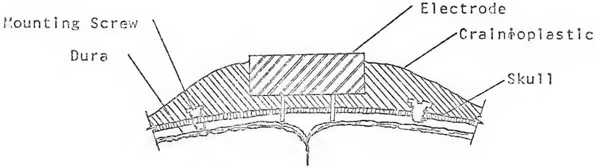

Figure 4-3 Implanted Electrode

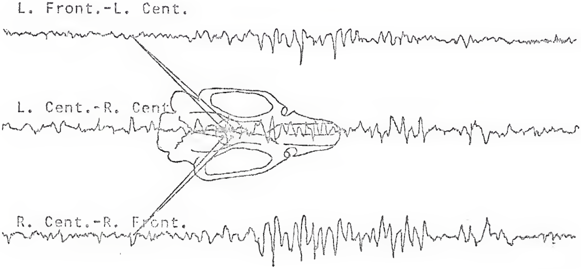

Figure 4-4 Electrode Configuration

Physiological Protocols

The analysis (in subsequent chapters) of the effects of abnormal electrical activity on the concentration of taurine in rat brain were based on four duplications of seven experiments. The protocols for each type of experiment are described below.

Control (C) For these experiments unimplanted animals were sacrificed in a manner to be used throughout all experiments in this protocol. The animal became accustomed to being dropped from small distances (i.e. six inches) several times before being dropped head first into a dewar of liquid Nitrogen and held there. This procedure was chosen over decapitation because of the apparent decrease in adverse effects with whole body freezing rather than decapitation followed by freezing of the head, Richter and Dawson(1948).

Control Implant (CI) These experiments were designed to determine whether or not the surgical procedures involved in implanting the EEG electrodes might cause a change in the intrinsic taurine level. The animals were sacrificed as in (C) after having been implanted with the electrodes and allowed to recover for a period of one week.

Pre-Seizure One (XS1) For all of the following experiments the animals were subjected to osmotic stressing by intraperitoneal injections of a mannose in water solution (1:1 weight: volume). The injections were given on a schedule of four percent of the body weight of the rat (volume:weight). This procedure is discussed in detail in Nevis and Thursby (1973). The injection time was considered as the starting time and then fourteen minutes later (approximately one half the time between the osmotic shock and the onset of the first seizure activity) the animals were sacrificed as in (C).

Seizure One (S1) These experiments were carried out by sacrificing the animals after the disappearance of the behavioral signs from the first epileptiform seizure.

Post-Seizure One(S1X) Each animal in this series was sacrificed four minutes after the cessation of abnormal behavioral signs. This corresponds to about one half of the interictal period.

Seizure Two(S2) These animals were sacrificed after the disappearance of the behavioral signs from the second seizure.

Post-Seizure Two(S2X) These experiments consisted of sacrificing the animals approximately four minutes after the second seizure activity was completed.

These protocols were designed to provide a set of data that reflects the change in the level of taurine in the brain that occurs concomitant with the changes in the level of abnormal electrical and behavioral activity. The behavioral activity was observed visually and the electrical activity was recorded from extradural electrodes implanted through the skull.

EEG Procedures

The rats were connected via a shielded cable to the amplifier-recorder system. The electrode configuration is shown in Figure 4-4. A typical recording is shown in Figure 4-5. With this montage we were able to observe the electrical activity of the cortex corresponding to primary motor and sensory areas. The electrocorticograms were recorded on a Grass model 78 Polygraph, the writing speed was 25 mm per second and initially the amplifier gains were set to cause a seven millimeter deflection with an input voltage of 50μV. The gain was then adjusted to give approximately a two millimeter deflection during the normal alert state. All animals whose EEG were recorded

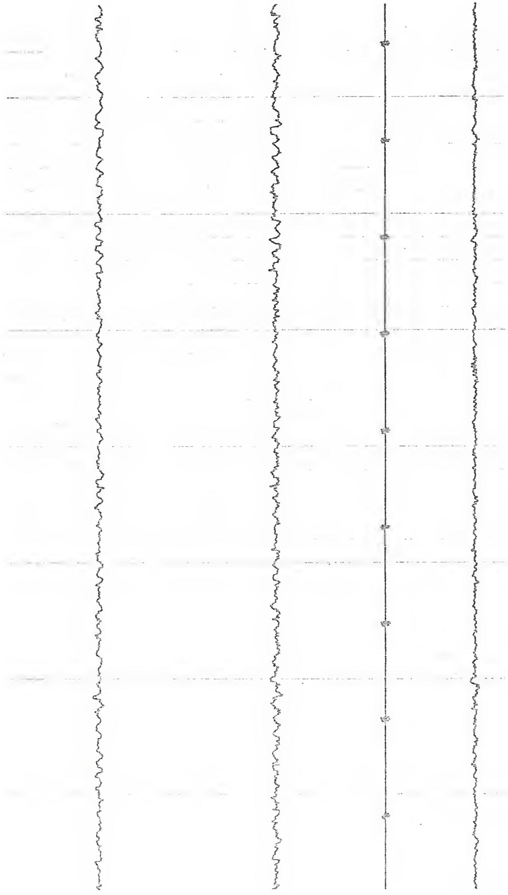

Figure 4-5 Typical "Normal" EEG Recording

underwent a control recording period of forty minutes prior to any osmotic stressing. In addition, as described above, a series of implanted controls were used to determine the effects of the implant on the chemical activity and to provide electrographic controls for further experimentation.

Excitability Scoring Criteria

The EEG recordings were then processed as described below, Thursby and Nevis(1973). The following is a description of the scoring criteria used to evaluate the EEG recordings for this dissertation. Each twelve second period was scored from zero to nine, then twenty-five periods averaged to give a mean score for each five minutes(300 seconds) epoch. These levels are:

Level Zero: (No overt seizure movements) No abnormal electrographic activity. This state may include normal alpha, beta, theta or "desynchronized" waveforms.

Level One: (No overt seizure movements) Less than one second of spikey burst in the alpha, beta, or theta frequency range, or one isolated spike with phase reversal or small amplitude sharp theta activity.

Level Two: (no overt seizure movements) Greater than one second but less than six seconds of spikey bursts in the alpha, beta or theta frequency range, or more than one spike, one second or less of single large amplitude delta waves, or twelve seconds of small amplitude sharp waves.

Level Three: (no overt seizure movements) Greater than six seconds of spikey bursts in the alpha, beta or theta frequency range or greater than one second of spike and waves(two to five waves per second frequency range), or a single fast burst of high amplitude (one hundred microvolts), alpha or greater than one but less than six seconds of high amplitude delta, or less than six seconds of burst supression or isoelectric response.

Level Four: (no overt seizure movements) Greater than one second of spike and waves, or greater than six seconds of single delta waves, or from five to ten seconds of burst supression, or isoelectric activity or twelve seconds of very spikey alpha, beta or theta activity, or ten seconds of spiking activity.

Level Five: Equivalent to Level Two with the addition of focal seizure movements (myoclonic jerk, clonus, automatisms).

Level Six; Equivalent to Level Three with focal seizure mivements.

Level seven; Equivalent to Level Four with focal seizure movements.

Level Eight: Equivalent to Level Four with generalized seizure movements(Tonic, tonic-clonic,clonic) for less than twelve seconds.

Level Nine: Generalized seizure movements for twelve seconds.

It should be emphasized that the above scoring algorithm is devised for experimental research studies in which very severely abnormal EEG patterns can be produced in a controlled manner. These rules are of course not directly applicable to clinical diagnostic evaluation in their present form.

Behavioral Scoring

In evaluating the overall neurologic state of an animal one must consider the behavioral activity as well as the electrical activity. Thus we also consider the following parameters: sleep, wakefulness and behavioral performance.

Normal movements

Walking is considered normal if the animal appears relatively relaxed and awake, by visual observation. All other normal animal movements are grouped together to include grooming, chewing, scratching and head movements. Some repetitive movements of chewing, grooming or scratching may indeed be abnormal, as discussed below.

Sleep and wakefulness

Wakefulness is subdivided for our work into two categories: awake and drowsey. The animal may drift into sleep from the latter classification and often it is difficult to evaluate except by typical sleep EEG activity.

Abnormal Movements

Abnormal movements include myoclonic jerks (single muscle jerks, either severe focal twitches or generalized single whole body jerks), focal seizure movements, which are repetitive and include local involvement of groups of muscles. This focal category may also include the "Jacksonian Motor March" (The Jacksonian motor march begins with focal repetitive activity (focal seizures) and spreads to other limbs or to the mouth and sometimes to the opposite side of the body. It may or may not become a generalized seizure). The most abnormal movement is that of the generalized seizure or "fit." This is characterized by wide spread muscular involvement of repetitive or sustained intense contractions. These may be constant contractions (tonic), or intermittent contractions (clonic), or mixed muscular contractions (tonic-clonic). It usually begins as a tonic motor activity and ends as clonic motor activity with mixed tonic-clonic activity interspersed throughout the seizure. These contractions may begin suddenly and symmetrically, or may develop from focal seizure activity as in the "Jacksonian March" described above.

Automatisms

Automatisms are another type of abnormal movements usually involving associated visceral motor or sensory activity, such as chewing, scratching, or teeth baring, or "hunching." These are repetitive and must be differentiated from normal chewing or scratching or other movements by their inappropriate and stereotyped appearance. These movements are considered normal, unless clearly associated with seizure activity.

For the total algorithm the animal is evaluated on movement, degree of wakefulness, and electrical activity.

For abnormal activity both electrical data and abnormal movement data are recorded on the excitability scale zero to nine, zero being no abnormal activity and nine being generalized seizure type waveforms and movements.

The evaluation of the EEG during large body movements of the animal may present a serious problem. The EEG is disrupted during this time and an accurate determination of the electrical activity of the brain is not available. At present it is evaluated as a level zero unless the movements are clearly epileptiform.

Analysis scheme

The entire data was first analyzed according to the scoring algorithm in twelve second epochs and scored for each epoch. These scores were in turn averaged over twenty-five epoch (five minutes). The data was then plotted and used for further evaluation of the excitability level of the electrical activity. The EEG score was used as an indicator of the severity of the seizure as well as a measure of the progress of the experiment.

Chemical Procedures

At the conclusion of each experiment the animal was frozen. The head was severed from the body and stored at zero degrees centigrade until chemical analysis could be carried out. To facilitate removal of the brain from the head the head was refrozen to liquid nitrogen temperatures at the time of extraction.

The head was split along the saggital plane and the brain was removed. The cerebellum was separated and stored for future analysis. The cerebellum was not included in this series of experiments as it was felt that the cortical activity would have a minimum effect on the cerebellar activity and by eliminating the large "constant" concentration of this section of the brain it would make the dynamics (if any) of the cerebrum more prominant. The cerebrum was placed in a cold pre-weighed homogenization tube and weighed to determine the tissue weight. A known amount of alpha amino-n-butyric acid(ABA) dissolved in trichloroacetic acid(TCA) was then added to the tube as an internal standard. In addition, enough TCA was added to bring the volume to approximately five ml. The tissue was homogenized at ice and water temperature for three minutes. The homogenate was transferred with five one-ml aliquots of TCA into two fifteen-ml centrifuge tubes. The material was then centrifuged at 3100 R.P.M. for five minutes. The supernate was withdrawn with a one-ml pasteur pipette and placed in two twenty-ml capped centrifuge tubes. Two ml of fresh TCA was added to each pellet of precipitate and the material was resuspended. This mixture was then recentrifuged as above for five minutes. The supernates were pooled and five ml of dry di-ethyl-ether was added to each tube. The tubes were then capped and shaken gently for five minutes. The sample was then centrifuged as above for five minutes. The lower aqueous phase was removed by pipetting and placed in a vacuum flask. The sample was frozen and evaporated to dryness. The residue was redisolved in 25.0 ml of 0.01 N HCl. This solution was filtered through a 0.47 micron filter. The filtrate was then refrigerated until analyzed on a JEOL model JLC-6AH automatic amino acid analyzer.

Analysis of the Chromatogram

The use of an internal standard alpha-amino-n-butyric acid (ABA) provides internal checks for losses due to spillage, error in dilution of the sample and volume differences of the injection aliquots on the column. The first procedure necessary in the use of internal standards is to pick the appropriate compound that does not occur at a detectable level in the tissue (brain) naturally. Using a "control" animal it was determined that ABA would be best for the standard, since its endogenous level did not produce a deflection on the chromatogram in the dilution that we would be using for the analysis. The concentration of the standard should be about the same as the species to be measured (assuming the molar response of the individual species is about the same). For this reason we picked a concentration of 2.5 micro-moles per milliliter of TCA using one ml aliquots per sample. Next, a standard curve was plotted expressing the relationship between the ratio of the area of the taurine peak to the area of the ABA peak versus the concentration of taurine. This relationship appears to be a straight line as shown in Figure 4-6. The line has a slope of 0.678 and a Y-intercept of 0.154.

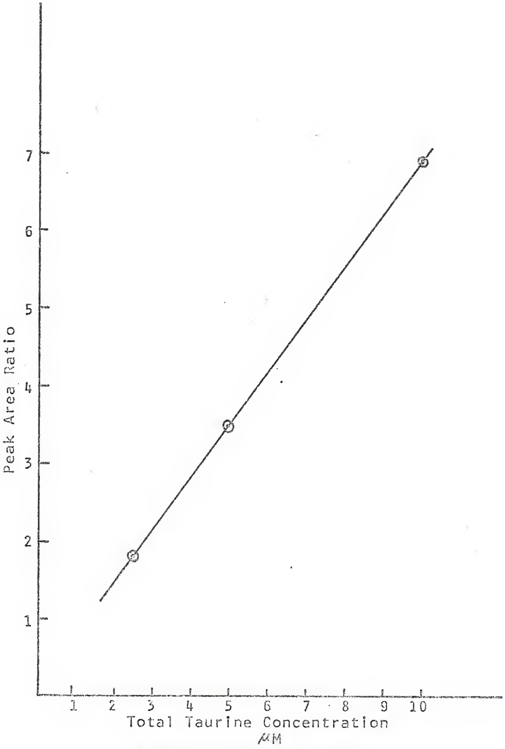

FIGURE 4-6 Standard Plot

CHAPTER FIVE

RESULTS

In this chapter the data that was derived from the experiments described in chapter four will be presented. In the following chapter the validity and implications of the data will be discussed.

EEG Data

Figure 5-1a,b,c and d, show the excitability scale plots for control experiments CI-1 through 4. This experiment was designed to test the variability of excitability between animals as well as the variability of the taurine level between "identical" animals. The overall average excitability (EEG) score for this set of experiments was 0.19. The next series of experiments was designated XS1-1 through 4. In this series the goal was to test the brain taurine content prior to the first seizure but after the administration of the osmotic agent (mannose). These results are presented in Figure 5-2a,b,c and d. The arrow on the x-axis is to indicate the time when the osmotic agent was administered. The first two

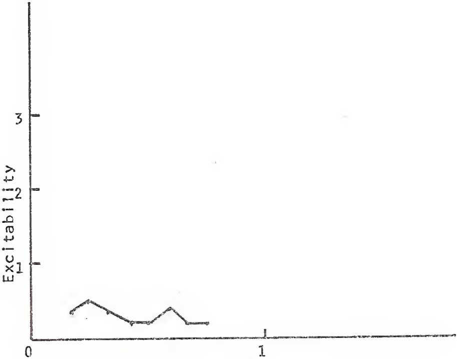

FIGURE 5-1a Experiment CI-1

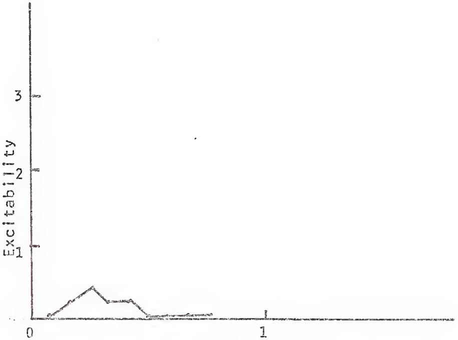

FIGURE 5-1b Experiment CI-2

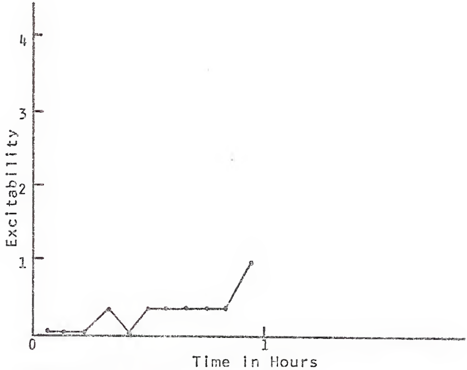

FIGURE 5-1c Experiment CI3

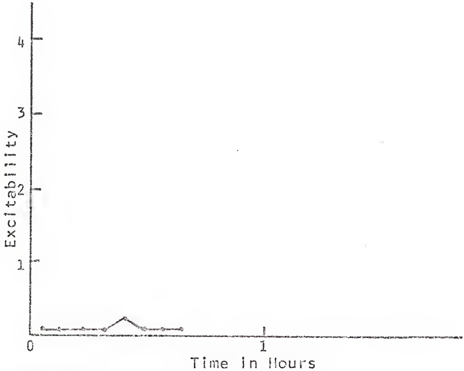

FIGURE 5-1d Experiment CI4

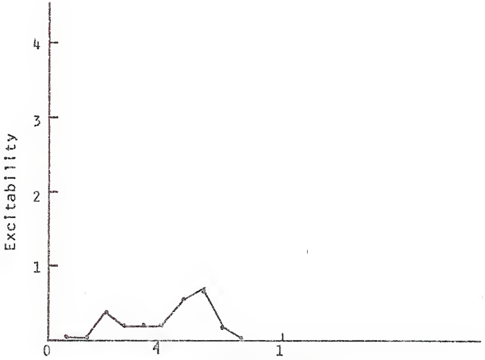

FIGURE 5-2a Experiment XS1-1

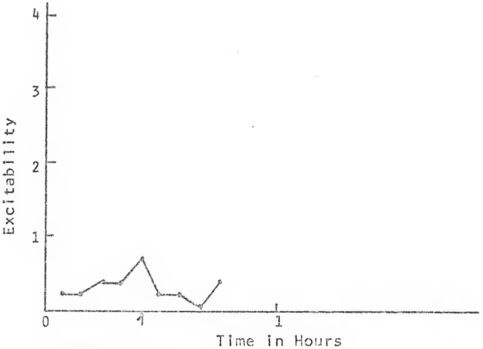

FIGURE 5-2b Experiment XS1-2

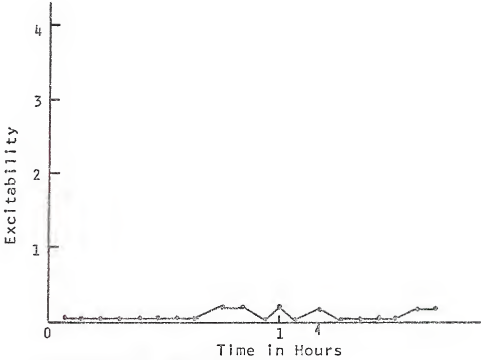

FIGURE 5-2c Experiment XS1-3

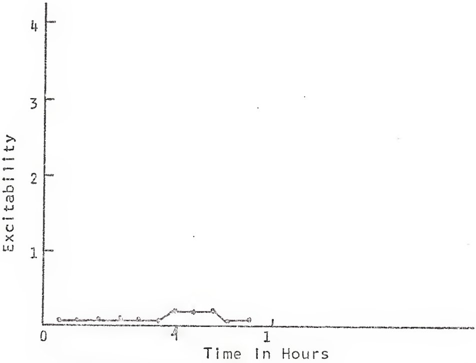

FIGURE 5-2d Experiment XS1-4

animals seemed to exhibit a higher excitability level; however, this is attributable to biological variations. The average score before the seizure was 0.13. The next series of experiments designated S1 Figure 5-3, were used to measure the level of taurine in the brain during the first seizure. The average pre-seizure level of excitability was 0.09. The actual peak levels during the respective seizures were 6.4, 3.56, 1.64 and 7.68. The seizures occured approximately thirty minutes after the administration of the osmotic agent. Figure 5-4a,b,c and d show the excitability results for the series of experiments designated S1X-1 thru 4. This set of experiments is designed to test the taurine level in the brain during the period following the first overt seizure. The animals were sacrificed four minutes after the seizure had ceased. The reduction of the excitability level is not evident simply because the time required to sacrifice the animal is less than one scoring epoch. The average pre-seizure excitability level was 0.33; the average peak level was 4.82. Again, seizures occured about thirty minutes after the administration of the mannose. In the next set of graphs, Figure 5-5a,b ,c and d, the excitability results for experiments S2-1 thru 4 are presented. The first seizure occured approximately thirty minutes after the injection of mannose and the second seizure occured about eight minutes after the first. The average excitability of these experiments prior to the seizure was 0.21 the average

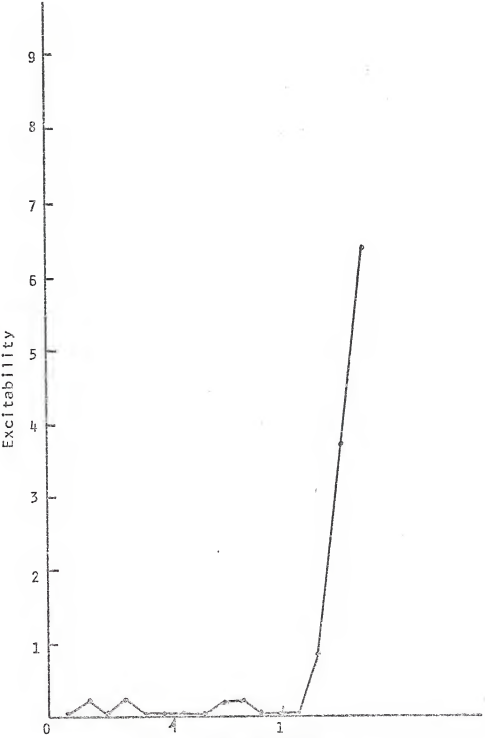

FIGURE 5-3a Experiment S1-1

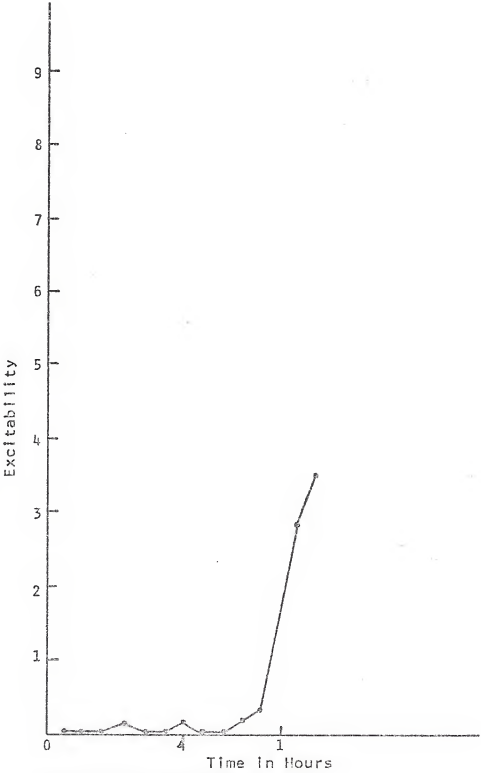

FIGURE 5-3b Experiment S1-2

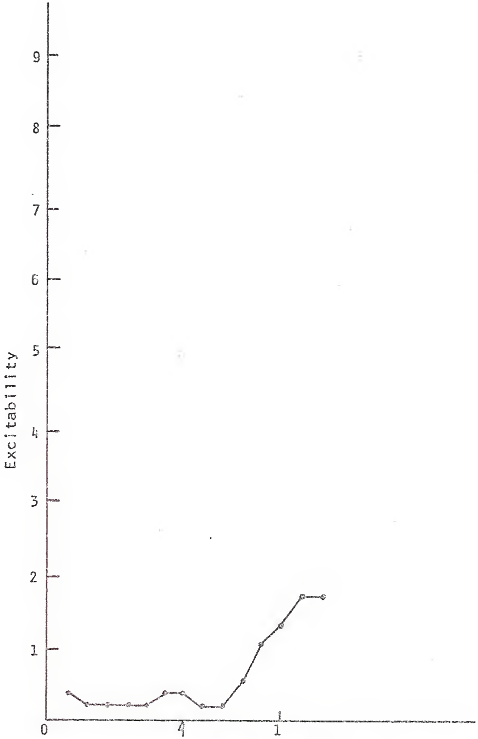

FIGURE 5-3c Experiment S1-3

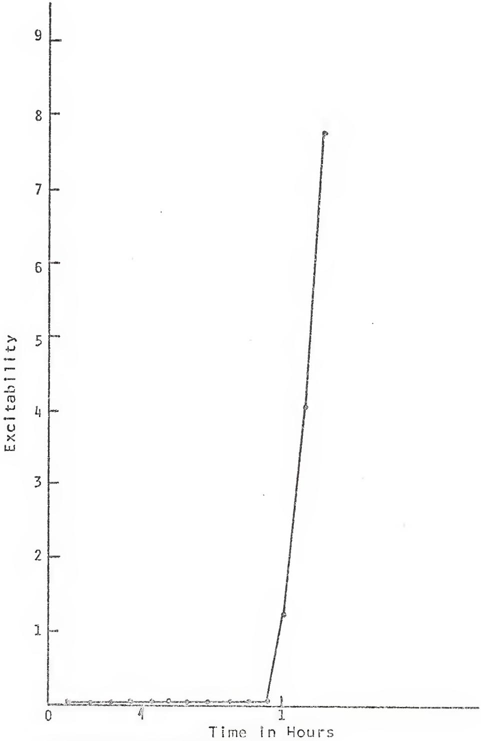

FIGURE 5-3d Experiment S1-4

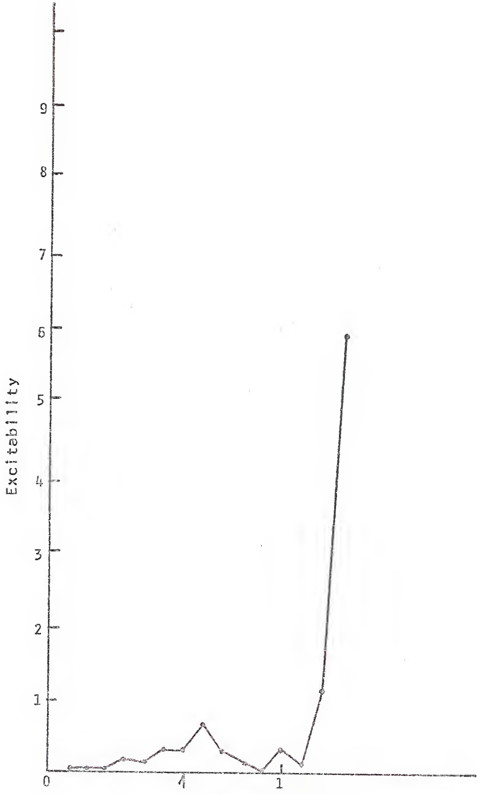

FIGURE 5-4a Experiment S1X-1

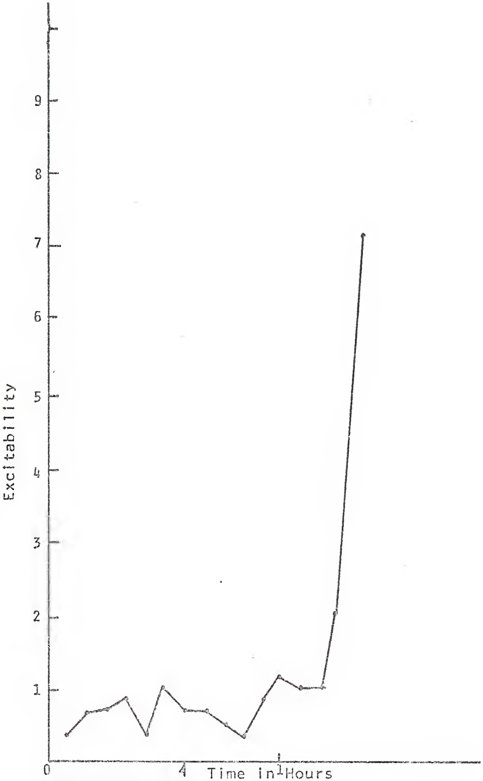

FIGURE 5-4b Experiment S1X-2

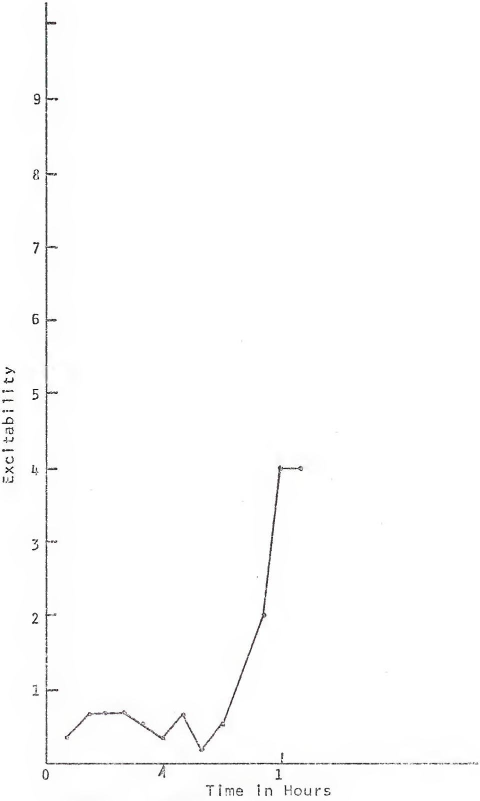

FIGURE 5-4c Experiment S1X-3

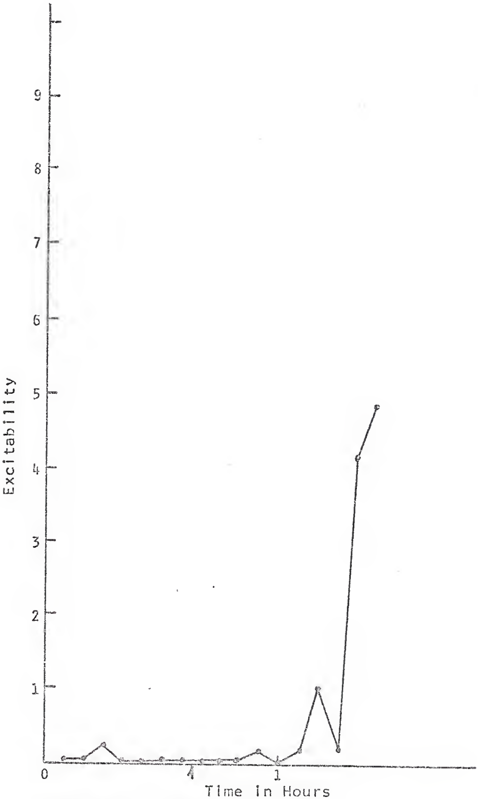

FIGURE 5-4d Experiment S1X-4

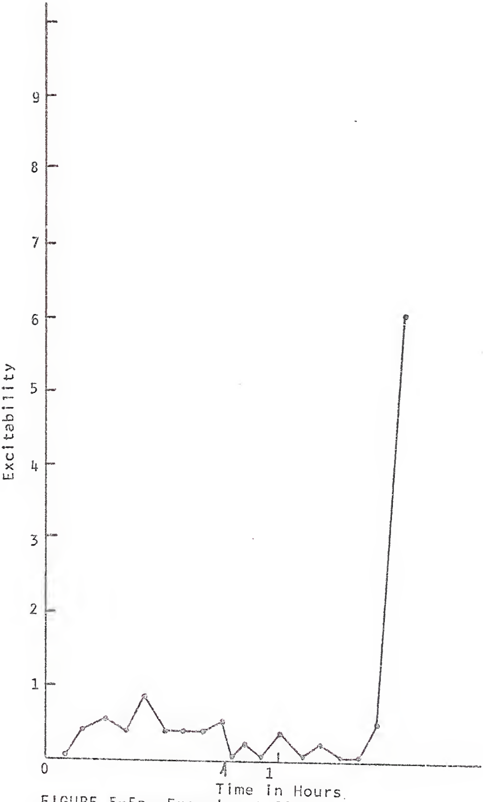

FIGURE 5-5a Experiment S2-1

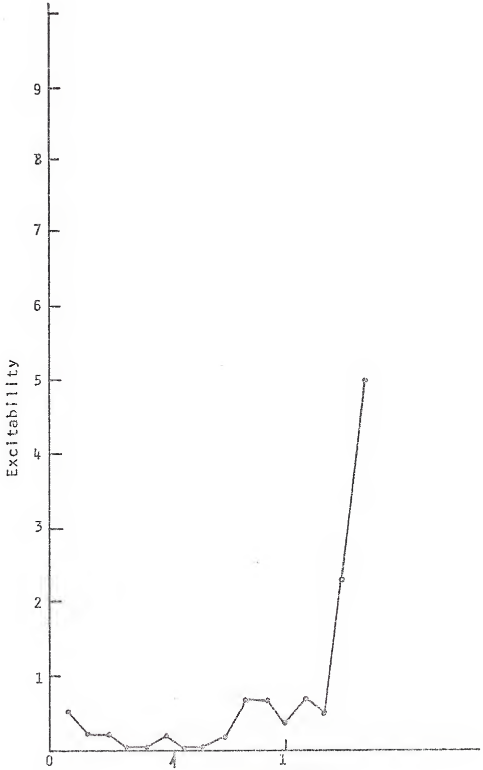

FIGURE 5-5b Experiment S2-2

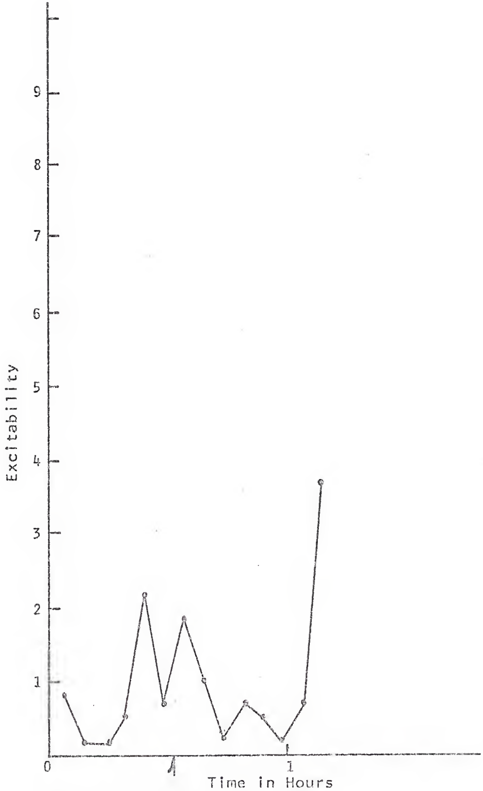

FIGURE 5-5c Experiment S2-3

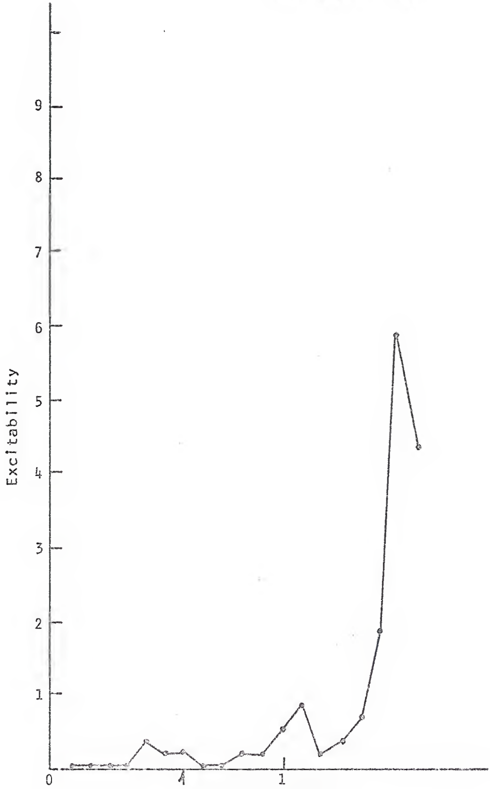

FIGURE 5-5d Experiment S2-4

peak level attained during the seizure was 5.12. These data showed the typical excitability response to the osmotic agent. For experiments S2X-1thru 4 the excitability levels are plotted in Figure 5-6a,b, c and d. They also show typical osmotic stressing response. The double peaking that is evident in two of the plots is due to the fact that the animals were allowed to experience two seizures prior to sacrifice. The peaking of the other two seizures is due to the averaging time that is used with this method. The average pre-seizure excitability level was 0.203, with an average peak seizure response of 4.9 on the excitability scale.

Figure 5-7 shows the excitability plot of an animal that underwent no overt seizure activity and only slightly abnormal electrical activity for a period of one hour and thirty minutes after the administration of the osmotic agent. Figure 5-8 shows the excitability plot of the animal that was observed to have undergone almost continuous seizure activity without the osmotic stimulus.

Figure 5-9 shows the relationship between the concentration of taurine in the brain and the progress of an osmotically induced seizure. The variability of the data is indicated by the standard deviation limits.

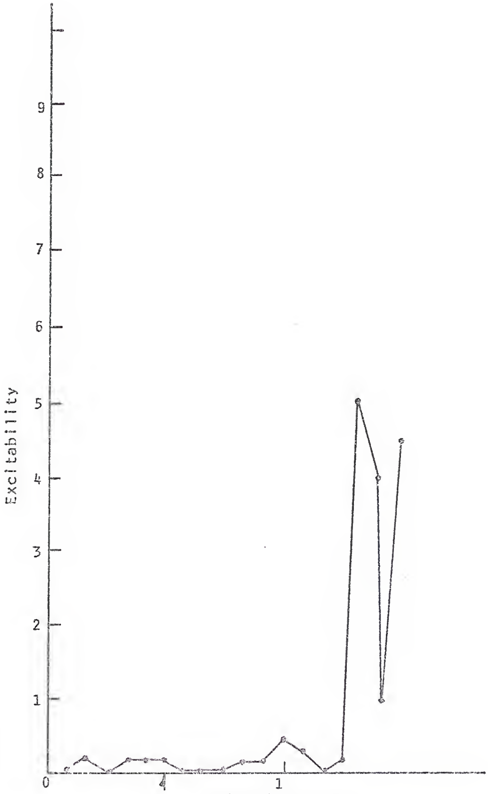

FIGURE 5-6a Experiment S2X-1

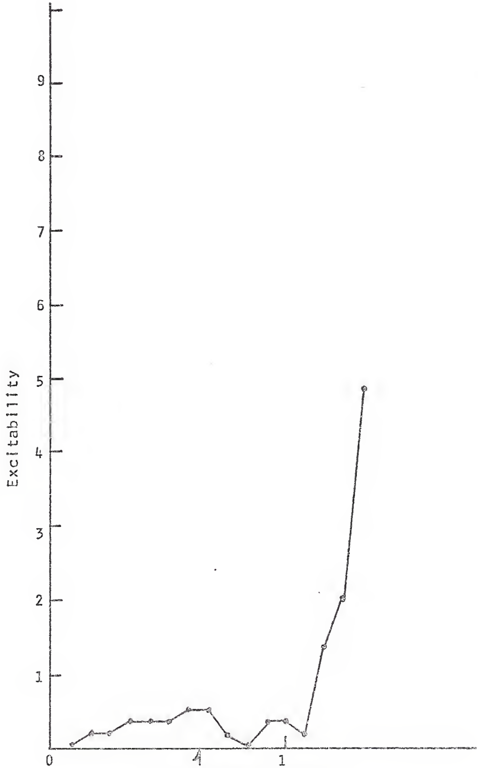

FIGURE 5-6b Experiment S2X-2

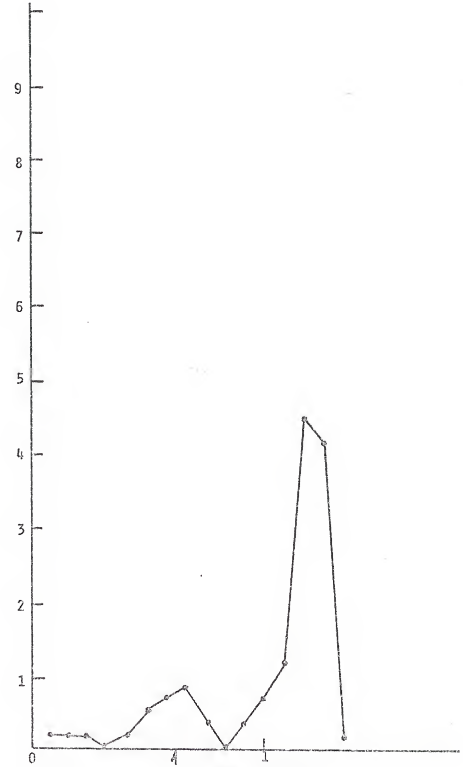

FIGURE 5-6c Experiment S2X-3

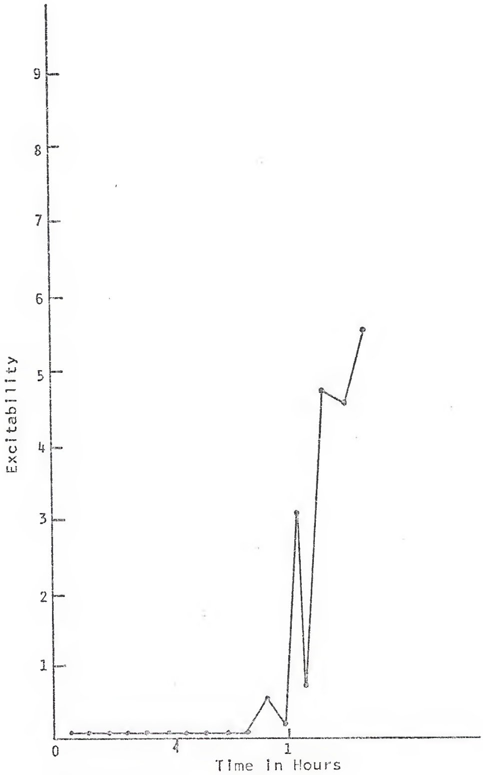

FIGURE 5-6d Experiment S2X-4

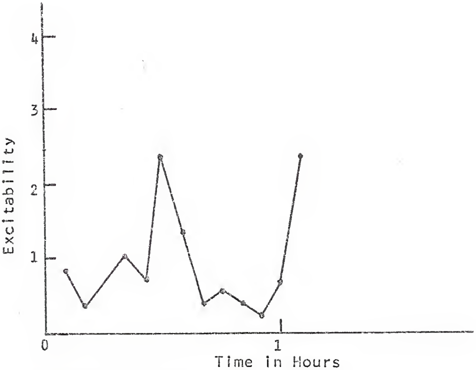

FIGURE 5-7 Status

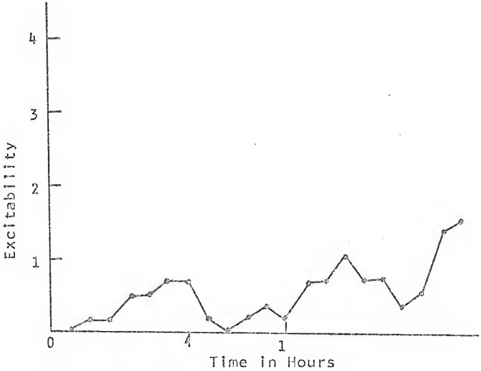

FIGURE 5-8 Experiment X-1

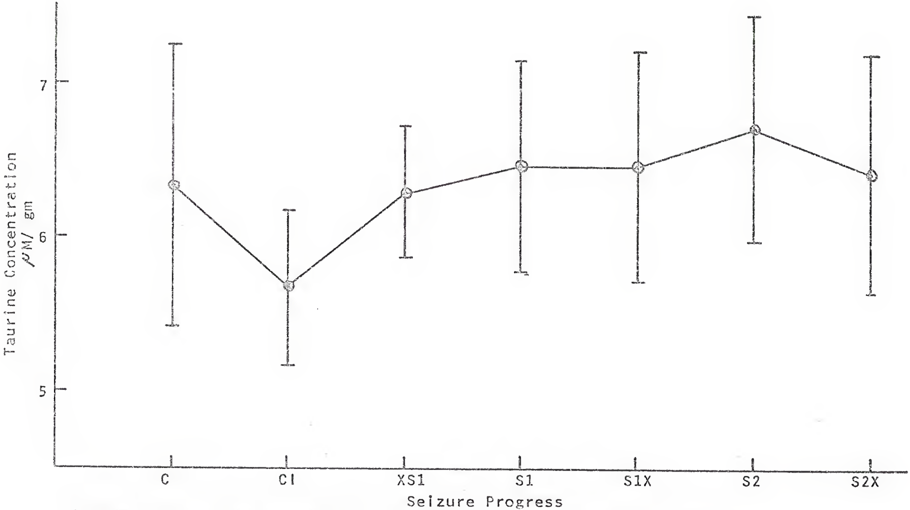

FIGURE 5-9 Taurine Concentration Versus Progress of Seizure Activity

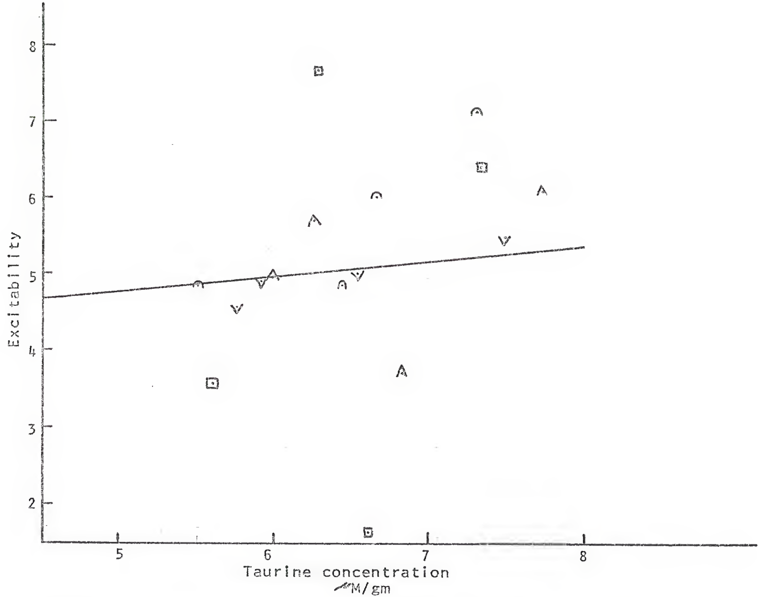

FIGURE 5-10 Peak Seizure Activity Versus Taurine Concentration

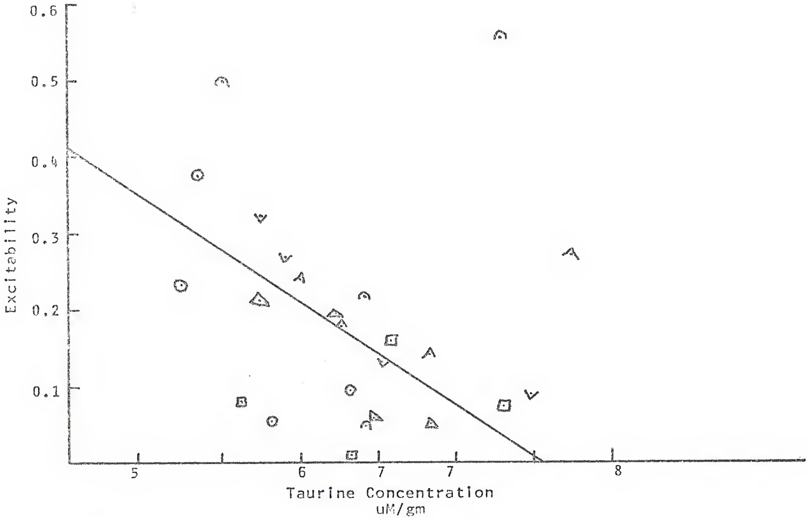

Figure 5-11 Average Pre-Seizure Activity versus Taurine Concentration

Figure5-10 shows a plot of the peak excitability of the individual experiments versus the concentration of taurine in the individual brains. The line plotted in this figure represents the linear regression line for the data. The correlation coefficient for the data is 0.07.

Figure5-11 is a plot of the average excitability level of the pre-seizure activity of the individual experimental animals versus the concentration of taurine in the brain. The line plotted in this figure represents the linear regression line for the data. The correlation coefficient for the data is 0.70. Table 5-1 summarizes the pertinent biological data for all experiments.

TABLE 5-1
COMPOSITE DATA TABLE

Experiment Type	Symbol	Animal Weight (gm)	Brain Weight (gm)	Taurine Conc. (uM/gm)	Pre-seizure Excit.	Peak Excit.
C-1		205	1.491	6.229		
2		228	1.370	5.862		
3		238	1.296	5.599		
4		293	1.181	7.646		
			Average	6.334		
CI-1	⊙	233	1.190	5.210	0.23	
2		235	1.012	6.326	0.09	
3		199	0.935	5.356	0.37	
4		235	1.210	5.816	0.05	
			Average	5.667	0.19	
XS1-1	◬	224	0.938	5.808	0.22	
2		235	1.027	6.217	0.19	
3		253	0.947	6.472	0.06	
4		300	0.891	6.836	0.05	
			Average	6.333	0.13	
S1-1	⊡	252	1.136	7.312	0.07	3.62
2		220	1.050	5.660	0.08	3.56
3		192	1.017	6.590	0.18	1.64
4		300	1.076	6.265	0.01	7.68
			Average	6.459	0.08	4.82

TABLE 5-1 (Cont.)
COMPOSITE DATA TABLE

Experiment Type	Symbol	Animal Weight (gm)	Brain Weight (gm)	Taurine Conc. (uM/gm)	Pre-seizure Excit.	Peak Excit.
S1X-1	∩	267	1.123	6.641	0.21	6.00
2		219	1.040	7.298	0.56	7.12
3		226	0.865	6.422	0.05	4.94
4		308	1.164	5.500	0.50	4.84
			Average	6.465	0.33	5.69
S2-1	∧	252	0.909	7.737	0.27	6.08
2		199	1.079	5.990	0.24	4.96
3		221	1.252	6.824	0.14	3.72
4		280	1.056	6.263	0.18	5.76
			Average	6.703	0.21	5.13
S2X-1	∨	310	1.338	6.525	0.13	5.00
2		221	1.093	5.902	0.27	4.90
3		277	1.085	5.757	0.32	4.56
4		242	0.886	7.481	0.09	5.48
			Average	6.416	0.20	4.98
X-1		196	1.064	6.672	0.54	
Status		189	1.322	7.308	0.86	

CHAPTER SIX

DISCUSSION AND CONCLUSIONS

The purpose of this chapter is to discuss and evaluate the evidence presented in the last chapter and to present reasonable conclusions based on the evidence. It should be pointed out that the method used for producing the seizures in these animals gave rise to EEG activity that would be considered extremely severe if seen in humans during intraictal periods, but which is comparable to that seen in humans during an acute seizures. The point of emphasis is that the scored EEG quantitates the severity of experimentally controlled seizures in animals, yielding maximum data in a minimum of time, yet reflecting an entire range of seizure activity. Clinical electroencephalography is more adapted to detecting very small changes in the EEG, and the possible presence of epileptic tendencies in a patient's EEG. Here we purposefully produced a series of severe seizures in a quantitatively controlled manner with a quantitative measure of each seizure. For this reason one should consider the results of this work only in terms of the severity of the seizures present.

It can be questioned whether the osmotic method of producing seizure activity does indeed produce epileptiform seizures or in fact some other manifestation of other pathologic changes. There is good evidence for the validity of this method of perturbation whether the route of administration is through the peritoneal cavity or through an intravascular route, Nevis (1970). In addition, the manifestations of the seizures both electrographically and behaviorally are so strikingly similar to human as well as other animal experimental epilepsies that there seems virtually no doubt that this method of experimental induction of epilepsy is indeed influencing the water and electrolyte balance of the brain to cause experimental epilepsies that are very close to the actual phenomena that are the basis for true epileptic attacks (Thursby and Nevis (1973) and Nevis and Thursby (1973)).

One might also question the validity of the method with respect to the possibility of the production of the seizure by some other unrelated link or mechanism, for example through anoxia or through formation of thromboemboli in cerebral vessels. Such an alternative is not supported since in four quite different types of experimentally induced epilepsies the effects of taurine on the severity of the seizure has been tested and are are indeed quite parallel. Thus the effects seen with the method used in this dissertation are identical to the effects seen when such convulsants as cobalt powder and Metrazol are used. The fourth type of induced seizure was that of electrically induced convulsions (Thursby and Nevis (1974)).

We have shown that the taurine level in rat brain does not change significantly during the progress of epileptic seizures. This data is similar to the data reported for glutamate, glutamine, GABA and alanine by King _et al._ (1974). There are several different reasons that could be proposed as an explanation of this phenomenon. First the level of taurine might not be important in the activity of the tissue during the seizure. This is highly unlikely but one must nevertheless consider the possibility. Secondly the effect of metabolism of the nervous system on the concentration of taurine may require many hours to respond to a given stimulus and effect a concentration change, one way or another. The mode of change of the endogenous level of taurine may require more than the few minutes that are required for the epileptic attack to run its course. Thirdly there may be a mechanism that carefully controls the level of taurine in the brain and, therefore, only extremely severe alteration of the biological system would lead to a change of the endogenous taurine level. Fourthly there may be a condition where, although there is no net change in the taurine level during the seizure, there might indeed be a large change in the turn over of taurine by increased anabolism in parallel with a increased catabolism of taurine at the same time. Finally the effect that taurine exerts on the membrane might be due to its ionic character in solution and not to its metabolic activity and thus there may be no need for the compound to be added to or removed from the system but instead taurine may simply be associated with or dissociated from a prosthetic group on the membrane. This association might be loose enough to allow taurine to be essentially free for chemical analysis.

There seems to be no simple correlation between the brain level of taurine and the peak level of the abnormal EEG activity. This indicates as was stated above that the actual seizure does not detectably affect the level of taurine in the brain. This could also support the hypothesis that there is no net change of taurine content in the brain. This may also be indicative of the fact that the peak level of abnormal electrical activity may not be the best measure of the total effect of the seizure.

In the present work there is good correlation between the level of taurine and the average level of the excitability plot prior to the seizure activity. This appears to point to the same concept that has been suggested by experiments on the effects of injected taurine on the seizure susceptability of different species in the animal kingdom (namely that higher taurine levels are accompanied by less epileptic activity). Indeed this suggests that the individual animal whose endogenous level of taurine is high show fewer effects from the surgery as manifested in slightly abnormal EEG activity. One could interpret this data slightly differently and say that the surgical procedures induced varying levels of abnormality into the system and that the taurine level reflected this change. The evidence for the first hypothesis is much stronger (i.e. the anti-convulsant activity). This would also be compatable with the data that indicates that taurine turnover is slow. Thus the effects of changing the level of taurine would be seen in a period of a few days or hours but not in just one hour. In other words, the mechanism by which taurine exerts its quieting force may require a relatively long period of time for its effect and thus the effects of the surgery could be controlled in the animals by endogenous level shifts of taurine over the post operative recovery period of one week. However, in the short term experiments where the excitability level was raised rapidly the time constant of the taurine action was too great to allow the taurine to affect the overall system response. This hypothesis is also strengthened by the fact that as mentioned earlier in the historical review there is evidence that the level of taurine does not change rapidly even after death. This hypothesis would suggest the need to seek a genetic variation in taurine metabolism which would most likely appear in a naturally occuring epileptic strain of mice or rats.

There is also the possibility that the mode of effecting the change in taurine level may be through absorption and release of taurine from the brain to the blood and total body system and vice versa. Urquhart *et al.* (1974) showed that there is an equlibrium established between the brain and the blood level of taurine in animals. This hypothesis would involve the blood brain barrier and its permeability to different species including taurine. Thus the glial cells of the brain may be responsible for the control of the brain levels of taurine. Considerable and detailed studies need to be done to clarify this point, including tracer studies.

Even though the level of taurine does not change during seizures in rats this does not preclude the possibility that another chemical species might change drastically during the seizure, and yet be related to taurine metabolism. For example, ISA may depend completely upon taurine for its origin but the change in ISA level need not be significantly reflected in the taurine levels. Tracer studies would be helpful here, also.

When this research was being conceived the first implication was that since taurine exerted a quieting force on the heart against dysrhythmia-inducing drugs like digitoxin there seemed to be a posibility that a similar effect could be seen with taurine in the brain. This hypothesis was not entirely without precedent as the use of diphenyl-hydantoin has been reported in cases of cardiac dysrythmias as well as its better established anti-epileptic effects on the abnormalities of the brain. This would seem to further indicate a fundamental role of taurine in stabilizing the membrane activity. The original motivation for the study of taurine was derived from a study of the anion deficit discovered in squid axoplasm thirty-five years ago, Schmitt and Bear (1939). Through many evolutionary eons mammals, including man, have evolved with high concentrations of taurine in their brains and it is presumably there for a reason. The fact that it is related to ISA and this in turn to the ionic milieu of the membrane and thus to the generation of the membrane potential leads one to speculate on the effects of these compounds in the nervous system. The antiepileptic effects of taurine show that there is a strong connection between taurine and the stability of the membrane and the nerve cell electrical activity.

There are clinical implications from the data presented here, in that one might be able to test the blood level of taurine and possibly predict the proclivity of a person to have seizures. This could be useful as a clinical tool in the identification of epilepsy and the detection of epilepsy in patients that may not show any of the behavioral signs, and indeed may be part of the basis for the fact that seizures are frequently seen in various amino acidurias. There is the posibility that the level of taurine may be involved in the phenomena of other cerebral dysrythmias such as Spreading Depression of Leao and thus possibly some cerebral migraine attacks. In general the identification and quantification of taurine in relation to known incidences of various epilepsies or other cerebral disorders may provide insight into the action of taurine in the brain.

This study, of course, does not provide an end to the investigation of the neuro-physiology of taurine but merely a beginning. There are many questions to be answered. Whether there is a strong correlation between the level of taurine and the abnormal electrical activity of the brain must be investigated by using many different types of experiments including tracer studies, deprivation studies, forced feeding studies, etc. Whether the level of taurine in the blood will reflect the brain level of taurine is an important question that has been only partly answered and a more detailed study should be carried out. The use of taurine in current clinical trials should indicate more as to the possibilities of taurine as an antiepileptic drug. In any event, the understanding of the nervous system and the neural membrane as a system must include the mechanisms of any and all compounds that relate to the stability of the membrane. It is encouraging in that there are thus additional posibilities of studying the cause and effect relationships that exist between the excitability of the membrane and the chemical environment of each cell. The fact that the presence of taurine in the brain is significant has been established by this study (and independently and concurrently by others in different areas of research). The fact remains that the true molecular action of taurine on the nerve cell membrane has yet to be delineated.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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